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ETHYLENE AND SOME ASPECTS OF METABOLISM OF SACCHAROMYCES CEREVISIAE

by

© KOLOTHUMANNIL CHANDAPILLAI THOMAS

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
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ABSTRACT

This project was undertaken to study the biogenesis of ethylene in yeast and effects of ethylene on yeast metabolism. Haploid strains of Saccharomyces cerevisiae (X-2180-1B and G13332) were used.

There was no net production of ethylene by the yeast growing in lactate medium. Glucose was found to induce synthesis of ethylene by the yeast. Cycloheximide inhibited glucose induced ethylene production; therefore, induction by glucose may involve de novo synthesis of proteins. Respiration deficient mutant yeasts growing in glucose medium produced large amounts of ethylene. This suggested that mitochondrial electron transport per se is not essential in the biosynthesis of ethylene. Oxygen was found to be stimulatory to the ethylene production by the yeast. Under anaerobic conditions the yeast produced some unidentified intermediate (or intermediates) which on admission of oxygen was rapidly converted to ethylene.

Methionine also stimulated production of ethylene by the yeast, but for maximal rate of production, glucose also was found to be necessary. Tracer experiments showed that radioactivity from L-methionine-U-^{14}C was incorporated into ethylene.

Ethylene production by the yeast was not affected by a change in pH between 4 and 6. Pyruvate inhibited the production of ethylene by the yeast. DL-Lactate, L-alanine and L-cysteine all convertible to pyruvate by yeast, also inhibited ethylene production. Addition of glucose to the yeast growing in lactate medium decreased the intracellular levels of L-alanine and increased the ethylene production.

Ethylene production by the yeast was stimulated by D-methionine, N-formylmethionine and L-ethionine.

Exogenously applied ethylene decreased uptake of glucose by the yeast. However, this inhibition by ethylene was preceded by an initial stimulation of glucose uptake by the yeast. Rates of respiration, ethanol production and $^{14}CO_2$ production from glucose-3,4-^{14}C, and intracellular levels of glucose-6-phosphate corresponded to the rate of glucose uptake by the yeast. Ethylene also caused increased uptake and retention of K^+ by the yeast.

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TABLE OF CONTENTS

PAGE

I. INTRODUCTION 1

II. LITERATURE REVIEW 3

  A. Biogenesis of Ethylene 3

    1. Ethylene production by cell free systems 3

    2. Subcellular localization of ethylene production. . 4

    3. Role oxygen in ethylene production 5

    4. Precursors of ethylene 5

    5. Effect of auxin on ethylene production 10

    6. Inhibitors of ethylene biosynthesis. 13

    7. Regulation of ethylene biosynthesis. 15

    8. Ethylene production by microorganisms. 18

  B. Physiological and Biochemical Effects of Ethylene. . . 19

    1. Auxin and physiological effects of ethylene. . . . 19

    2. Effect of ethylene on respiration. 21

      a. Mechanism of stimulation of respiration by ethylene. 21

    3. Effect of ethylene on membrane permeability. . . . 23

    4. Interaction of carbon dioxide and ethylene 23

    5. Effects of ethylene on yeast. 25

III. MATERIALS AND METHODS. 27

  A. Materials. 27

    1. Chemicals 27

    2. Yeasts 27

Page

B. Methods 27

1. Maintenance of the yeasts 27

2. Cultivation of the yeasts for the study of ethylene biosynthesis and biochemical effects of ethylene 28

3. Isolation of respiration deficient mutants . . . 29

4. Starvation of the yeast 29

5. Anaerobic cultivation of the yeast 29

6. Cultivation of the yeast in presence of applied ethylene 30

7. Collection of ethylene 30

8. Determination of ethylene by gas chromatography. . 31

9. Determination of the radioactivity of ethylene produced from L-methionine-U-^{14}C 32

10. Measurement of Respiration 32

a. Warburg method 32

b. Biological oxygen monitor method 32

11. Determination of growth rate 33

12. Determination of dry weight 33

13. Determination of ethanol 33

14. Determination of ethanol production by yeast in presence of metabolic CO_2 and applied ethylene . . 33

15. Determination of glucose uptake 34

16. Determination of 3-O-methylglucose uptake 35

17. Determination of $^{14}CO_2$ production from glucose-3,4-^{14}C. 35

18. Preparation of yeast extract and assay of enzymes 36

19. Presentation of enzyme activity 38

Page

20. Determination of glucose-6-phosphate ATP and ADP and L-alanine 38

21. Determination of K^+ uptake........................ 39

IV. RESULTS AND DISCUSSION.................................. 40

A. Production of Ethylene by *Saccharomyces cerevisiae* 40

1. Ethylene production by *Saccharomyces cerevisiae* grown in lactate medium..................... 40

2. Induction of ethylene production in yeast by glucose..................................... 42

3. Effect of glucose concentration on induction of ethylene production..................... 48

4. Effect of oxygen on ethylene production...... 51

5. Effect of pH on ethylene production.......... 56

6. Effect of L-methionine on ethylene production. 60

7. Synergistic effect of D-glucose and L-methionine on ethylene.................................. 60

8. Changes in the pH of the medium, cell mass and rate of ethylene production................. 68

9. Effect of L-methionine on production of ethylene by the filtrate of glucose-grown yeast...... 72

10. Effect of L-methionine concentration on ethylene production by yeast....................... 72

11. Effects of D-methionine and L-ethionine on ethylene production by yeast....................... 78

12. Inhibition of ethylene production by pyruvate. 84

13. Effects of L-alanine and L-cysteine on ethylene production by yeast....................... 87

14. Role of glucose in the stimulation of ethylene production................................ 93

15. Effect of β-alanine on ethylene production by yeast.................................... 99

Page

16. Production of ethylene by adenine- and methionine-requiring mutant yeast.................. 104

17. Production of ethylene from L-methionine-U-^{14}C... 108

18. Effect N-formylmethionine and methionine-containing dipeptides on ethylene production by methionine-requiring yeast.................... 108

19. Production of ethylene from methional........... 114

B. Effects of Ethylene on Yeast......................... 117

1. Ethylene and respiration of yeast................ 117

    a. Respiratory activity of yeast grown in the presence of ethylene......................... 117

    b. Respiration of the yeast starved in the presence of ethylene......................... 121

2. Effects of ethylene of glucose uptake and ethanol production by yeast............................. 127

3. Ethanol production from glucose by yeast starved in the presence of ethylene....................... 127

4. Effect of metabolic CO_2 and exogenously applied ethylene on ethanol production from glucose by yeast.. 133

5. Effect of ethylene on $^{14}CO_2$ production by yeast from glucose-3,4-^{14}C............................ 140

6. Effect of ethylene on uptake of glucose by yeast.. 144

7. Effect of ethylene on synthesis of glycolytic enzymes... 150

8. Effect of ethylene and glucose on intracellular levels of glucose-6-phosphate and adenosine triphosphate....................................... 153

9. Effect of ethylene and carbon dioxide on K^+ transport in yeast................................ 156

V. GENERAL DISCUSSION AND CONCLUSIONS 163

VI. BIBLIOGRAPHY.. 171

LIST OF TABLES

Table Page

1. Production of ethylene by S. cerevisiae (X-2180-1B) grown in lactate medium 41
2. Effect of cycloheximide on glucose-induced ethylene production 47
3. Ethylene production by respiration deficient mutants 52
4. Effect of pH on ethylene production 57
5. Changes in pH, cell mass and rates of ethylene production on addition of glucose and L-methionine 70
6. Effect of L-methionine, D-glucose, D-glucose oxidase, catalase, and H_2O_2 on ethylene production by yeast 100
7. Effect of carbon source on the intracellular levels of free L-alanine in yeast 101
8. Production of radioactive ethylene from L-methionine-U-^{14}C by S. cerevisiae (G1332) 107
9. Effects of L-methionine, N-formyl-L-methionine and methionine-containing peptides on ethylene production by S. cerevisiae (G1332) 113
10. Production ethylene from methional 115
11. Intracellular levels of ATP and ADP in S. cerevisiae (X-2180-1B) grown in presence and absence of added ethylene 120
12. Effects of metabolic CO_2 and exogenously applied on rate of ethanol production from glucose by S. cerevisiae (X-2180-1B) 137
13. Effect of 100 ppm ethylene in air on uptake and metabolism of glucose-3,4-^{14}C by S. cerevisiae (X-2180-1B) 143
14. Effect of growing S. cerevisiae (X-2180-1B) in the presence of 100 ppm ethylene in air on synthesis of certain enzymes 151

LIST OF FIGURES

Figure		Page
1.	Effect of glucose on ethylene production	44
2.	Effect of cycloheximide on glucose-induced ethylene production	46
3.	Effect of glucose concentration on ethylene production	50
4.	Ethylene production by anaerobically grown yeast	55
5.	Effect of pH on ethylene production	59
6.	Production of ethylene by yeast in presence of glucose or L-methionine	62
7.	Synergistic effect of glucose and L-methionine on ethylene production (methionine added first)	65
8.	Synergistic effect of glucose and L-methionine on ethylene production (glucose added first)	67
9.	Ethylene production by cell-free growth medium	74
10.	Effect of L-methionine concentration on ethylene production	77
11.	Effect of D-methionine on ethylene production	80
12.	Effect of L-ethionine on ethylene production	83
13.	Effect of pyruvate on methionine-stimulated ethylene production	86
14.	Inhibition of ethylene production by pyruvate	89
15.	Effects L-alanine and L-cysteine on ethylene production	91
16.	Effects of catalase and glucose oxidase on ethylene production by yeast	96
17.	Effects of catalase and glucose oxidase on ethylene production by filtrate	98
18.	Effect of β-alanine on ethylene production	103
19.	Production of ethylene by adenine- and methionine-requiring yeast	107

Page

20. Effects of L-methionine, N-formylmethionine, L-methionylglycine and L-methionylmethionine on ethylene production 112

21. Respiration of yeast grown in presence of ethylene 119

22. Effect of starving on respiration of yeast 123

23. Effect of glucose concentration on the respiration of previously starved yeast 125

24. Glucose uptake and ethanol production by yeast 127

25. Ethanol production from glucose by previously starved yeast 132

26. Rate of ethanol production by yeast in presence of ethylene 135

27. Rate of ethanol production. Percentage of control 139

28. $^{14}CO_2$ production from glucose-3,4-^{14}C 142

29. Effects of ethylene on glucose uptake 146

30. Effects of ethylene on 3-O-methyl glucose uptake 151

31. Effects of glucose and ethylene on intracellular levels of glucose-6-phosphate 155

32. Effects of glucose and ethylene on intracellular levels of ATP 158

33. Effect of CO_2 and ethylene on K^+ uptake 161

I. INTRODUCTION

Although the importance of ethylene in plant biology is well recognized, its biogenesis and its mode of action are not clearly understood. Various compounds as possible precursors of ethylene have been suggested. L-Methionine is thought to be the most important precursor in plants, although the role of precursor of ethylene has been assigned to various other compounds.

The pathway of conversion of methionine to ethylene is a subject of much controversy. Suggestions have been made that L-methionine is first transaminated to yield the α-keto analogue of methionine and this compound in turn is converted to ethylene by a free radical mechanism (108). There is evidence for and against this proposal. S-adenosyl methionine is also thought to be a possible intermediate in the conversion of methionine to ethylene (26). But there is no experimental evidence in support of this proposal.

According to Burg (26), mitochondrial electron transport is an essential requirement for the biosynthesis of ethylene. He states that, "For methionine to be converted to ethylene, energy supplied by mitochondria appears to be required and electrons released from methionine have to be carried by a cofactor to the electron transport system." There is no direct experimental evidence in support of this proposal.

At least one step in the conversion of methionine to ethylene is thought to be oxygen-dependent. According to Baur _et al_. (15), methionine is first converted to an unidentified intermediate, followed by an oxygen dependent reaction in which the intermediate is converted to ethylene.

The roles of oxygen and mitochondrial electron transport in the biosynthesis of ethylene can be studied under well defined conditions if yeast is used as a test organism. The ability of the yeast to grow under anaerobic conditions offers distinct advantages over plants in the study of ethylene biogenesis. The use of respiration deficient yeasts (which have no functional mitochondria) could tell whether mitochondrial electron transport is essential for ethylene biosysnthesis.

It is difficult to study the role of glucose in the biosynthesis of ethylene in higher plants, since plant materials usually contain large amounts of the free sugar. Yeast, on the other hand, can be grown in non-glucose media, and the effect of glucose on ethylene production can be studied by addition of the sugar to the growth medium.

It has been reported that ethylene does not have any effect on yeast physiology (2). This kind of report is perhaps not surprising since yeast under normal growth conditions produces large amounts of ethylene, and therefore, exogenously applied ethylene may not show any apparent effect on yeast physiology. But if the synthesis of ethylene by the yeast could be controlled by manipulation of growth conditions, it might be possible to study the effect of ethylene on the physiology of yeast.

II. LITERATURE REVIEW

A. BIOGENESIS OF ETHYLENE

1. Ethylene Production by Cell-free Systems

The elucidation of the mechanism of ethylene biogenesis has been complicated by the fact that ethylene is produced in significant quantities by non-enzymatic (not necessarily non-biological) means in many tissue extracts. Nevertheless, attempts have been made to isolate cell-free systems that are capable of producing ethylene from known precursors. Stinson and Spencer (169) reported the successful isolation of a soluble enzyme system that could convert β-alanine to ethylene. However, the ethylene production by the same enzyme preparation was stimulated to a greater extent by L-methionine than by β-alanine (171).

Formation of ethylene from methional by cell-free extracts of cauliflower florets was demonstrated by Mapson and Wardale (113). Ethylene production from methionine and its α-hydroxy analogue by such a cell-free extract was low and for maximum production both particulate and non-particulate fractions of the tissue were required.

Lynch (95) reported that the soil fungus Mucor hiemalis produced ethylene from methionine in the presence of glucose; the filtered cell-free growth medium produced 60 times more ethylene than the unfiltered fungus-containing medium. Uninoculated medium did not produce any detectable amount of ethylene.

In the light of these observations, it is doubtful whether cell integrity is an essential requirement for ethylene biosynthesis. However, most of the workers were not successful in obtaining a cell-free system which could convert methionine to ethylene (2, 26). The enzyme system described by Stinson (171) was non-specific as to the requirement of substrates, and it could convert both β-alanine and L-methionine to ethylene.

2. Subcellular Localization of Ethylene Production

The site of ethylene production is as yet unknown. Subcellular organelles as possible sites of ethylene biosynthesis have received much attention. Thus ethylene production by chloroplasts (139) and mitochondria (118, 119, 120, 143, 164, 165, 170) has been reported. Since exposing mitochondria to 0° to 100° did not prevent ethylene production (165) it is possible that the precursors of ethylene are present in mitochondria and their conversion to ethylene under these conditions, is probably not enzymatic. Stinson and Spencer (170) observed that there was a relationship between the loss of mitochondrial integrity and the rate of ethylene production.

Ku and Prat (80) could not get any significant amount of ethylene from active mitochondria suspended in a buffer containing 0.3 mM ATP and 0.25 M sucrose. They concluded from this study that active mitochondria do not produce any ethylene. However, the experimental conditions these authors used did not seem to favor ethylene production. For example, sucrose at high concentrations, is known to inhibit ethylene production (85). Also, suspension of an active preparation of mitochondria in a buffer containing 0.3 mM ATP, resulted in reduction of the rate of oxygen uptake to the basal level (the energized state), and it is reported that molecular oxygen plays an active role in the biogenesis of ethylene (29, 111). Thus the lack of ethylene production by active mitochondria observed by these authors (80) might result from the inhibitory effect of sucrose and excess of ATP present in the medium. It is interesting to note that 'aged' mitochondria which are not tightly coupled and therefore cannot be influenced by the presence of an excess of ATP, produce large amounts of ethylene (38).

3. Role of Oxygen in Ethylene Production

Ethylene production is thought to be an aerobic process (28, 111). Anaerobic conditions do not favor the production of ethylene. Thus, there is a great decrease in the amount of ethylene produced from L-methionine and its α-keto analogue by apple tissue in a nitrogen atmosphere (15). If anaerobically treated tissues are brought to aerobic conditions, there is an accelerated synthesis of ethylene, and it is suggested that under anaerobic conditions some precursor of ethylene accumulates, which on admission of oxygen is rapidly converted to ethylene (29). Baur _et al_. (15) have shown that at least one of the steps in the conversion of methionine to ethylene is oxygen dependent. Even though the stimulatory effect of oxygen on ethylene production is a recognized fact, it is still not clear whether there is an absolute requirement for oxygen. Studies involving microorganisms which can grow under anaerobic conditions might provide an answer regarding the role of oxygen in the ethylene biosynthesis. If molecular oxygen _per se_ is required for ethylene biogenesis, then there appears to be no recognition of this fact in the present proposed biochemical pathways.

4. Precursors of Ethylene

A number of substances have been proposed as possible precursors of ethylene in biological systmes. Among them are β-alanine (118, 169. 177), linolenic acid (88), glucose (33, 34, 136, 158), acetate and other tricarboxylic acid cycle intermediates (58), ethanol (136), and methionine (3, 16, 28, 55, 57, 86, 88, 113). Excellent reviews of the research work that led to the proposal of such an array of possible precursors have been written (1, 2, 134, 163). Methionine has been investigated extensively, and there is a large body of support for it being an important precursor.

L-methionine and several structurally related compounds have been implicated as possible substrates and intermediates of ethylene biosynthesis. The ability to form ethylene from such compounds is dependent on the presence of the structure $R\text{-}S\text{-}CH_2\text{-}CH_2\text{-}$, where the R group is either CH_3 or C_2H_5 but not H (Homocysteine is completely inactive). Removal of sulphur from the molecule as in homoserine ($HO\text{-}CH_2\text{-}CH_2\text{-}CH(NH_2)\text{-}COOH$) or shortening the carbon chain as in S-methyl cysteine ($CH_3\text{-}S\text{-}CH_2\text{-}CH(NH_2)\text{-}COOH$) or blocking the sulphur atom as in methionine sulphoxide or sulphone prevented the conversion to ethylene (108). Lieberman et al. (86) suggested that ethylene was derived from 3 and 4 carbon atoms of methionine. When labelled methionine was supplied to apple, banana, or pea stem sections, incorporation of label into ethylene was observed; only when carbons 3 and 4 labelled was the resulting ethylene labelled (28). Carbon atoms in 1 and 2 positions were liberated as carbon dioxide and sulphur and methyl carbon were retained in the tissue. In one experiment with methionine at a level of 2.4×10^{-7} M, at which concentration no great stimulation of ethylene production was observed, 80% of the $C_3\text{-}C_4$ moiety of the methionine was converted by apple slices to ethylene within one hour (108).

Although it is fairly well established that methionine is an important precursor of ethylene in plants (2), the pathway of conversion of methionine to ethylene is not well understood. Several proposals based mainly on model studies have been made (185). Since methional and 2-keto-4-thiomethylbutyric acid (KMBA) yielded ethylene with considerable ease in model systems, these two compounds were thought to be possible intermediates in the conversion of methionine to ethylene. In fact, a cell-free enzyme system which can convert methional to ethylene has been isolated (113). The conversion of methional to ethylene required the presence of two enzyme systems, the first generating peroxide and the second catalyzing the conversion of methional to ethylene in the presence of peroxide. The enzyme generating hydrogen peroxide appeared to be similar to fungal glucose oxidase, for like the latter, it was highly specific for its substrate D-glucose (112). A heat stable cofactor was also found to be necessary for the enzymatic cleavage of methional to ethylene, and this was identified as methanesulphinic acid (110). Suggestions have been made that the methional cleaving enzyme is a peroxidase (106, 110). In spite of the overwhelming evidence that methional can yield ethylene under a variety of conditions in vitro, it is not yet proven whether this compound is a normal intermediate in the conversion of methionine to ethylene in vivo. The enzyme preparation reported by Stinson (171) could convert methional to ethylene and the conversion was found to be non-enzymatic in nature, since the heat denatured enzyme preparation was far more active in converting methional to ethylene than the unheated enzyme.

It has been proposed that methionine is first transaminated, probably with phenylpyruvic acid as amino acceptor (79, 107). The resulting KMBA is converted to ethylene by a peroxidase enzyme in the presence of H_2O_2 and certain phenolic compounds. Mapson et al. (109) observed that KMBA promoted ethylene production when added to cauliflower tissue and was converted more efficiently into ethylene than methionine. In addition, unlabelled methionine increased incorporation of ^{14}C-KMBA into ethylene, while in the reverse experiment unlabelled KMBA decreased incorporation of ^{14}C-methionine into ethylene.

A specific aminotransferase that catalyzes the transfer of amino groups from L-methionine to give KMBA has been isolated from germinating peanut seed and partially purified, by Durham _et al_. (49). These authors suggested that the KMBA thus formed could readily be converted to ethylene by a free radical mechanism. Pyruvate was the most effective acceptor of the amino group; pyridoxal-5-phosphate and reducing agents were required for maximum activity.

It is not certain whether KMBA is a normal intermediate in the conversion of methionine to ethylene inside the cell. Lieberman and Kunishi (85) reported that the stimulation of ethylene production by cauliflower tissue in buffer solution containing KMBA, observed by Mapson _et al_. (109), did not result from the activation of natural _in vivo_ system. The increased ethylene production was a result of an extracellular ethylene-forming system that leaked from cauliflower tissue and caused the degradation of KMBA. This exogenous ethylene-forming system was similar to the ethylene-forming system described by Yang (186). Lieberman and Kunishi (85) concluded that KMBA was probably not an intermediate in the biosynthetic pathway between methionine and ethylene. Chemical and kinetic studied made by Baur _et al_. (15) and Yang and Baur (185) also supported the view that KMBA is not a possible intermediate of ethylene synthesis from methionine. These authors found that ethylene was produced from methionine at a faster rate than from KMBA. In the light of these lines of evidence, the involvement of KMBA or an amino transferase that converts methionine to KMBA in the conversion of methionine to ethylene is doubtful.

Burg and Clagett (28) and Burg and Burg (30) suggested S-adenosylmethionine as a possible intermediate in the biosynthetic pathway between methionine and ethylene. They based their argument mainly on the observation that S-adenosylmethionine was formed in good yield when ^{14}C-methionine was applied to apple slices. Also, this compound has a tendency to split-off its S-methyl group and be converted to ethylene by the Cu^+-ascorbate model system. If such a system exists in living tissue one should expect a close relationship between the ethylene production and the activity of methionine adenosyl transferase enzyme. No such information is forthcoming.

An interesting observation which did not receive much attention from research workers engaged in the study of ethylene in plant biology was made by Demorest and Stahman (46). These workers found that peptides containing methionine at the C-terminal position were far more active in a model system in producing ethylene than free methionine. Peptides that contained an N-terminal (L-met-gly-gly) or internal (gly-L-met-gly) residue of methionine produced little or no detectable ethylene. In addition, N-acylated derivatives of methionine like N-formyl-DL-methionine and N-acetyl-DL-methionine were equally, and much more active, respectively, in producing ethylene, than peptides with a C-terminal methionine derivative. These results suggested that proteolysis in the plant could increase ethylene production by producing peptides with a C-terminal methionine residue. This possibility was confirmed by the finding that limited proteolysis of crystallized egg albumin resulted in a significant ethylene production by the model system.

The acylated derivatives of methionine as possible intermediates of a biosynthetic pathway between methionine and ethylene have not received serious attention from research workers in this field. It is known that acylase activity towards N-acetyl-DL-methionine is high in various plants compared with acylase activity towards other N-acetylated and N-aclylated amino acids (39). Keglevic et al. (73) have found that N-malonyl methionine and not 2-keto-4-thiomethylbutyrate is the major acidic metabolite of D-methionine in intact tobacco plants. Thus N-acylated derivatives of methionine are involved in the metabolism of methionine in some plants and may be precursors of ethylene.

There is only one report where N-acetylmethionine was tried as a precursor for ethylene production in vivo (26). It was found that N-acetylmethionine in the concentration range of 10 to 100 mM profoundly inhibited ethylene synthesis in apples. Details of that experiment are not available, and it may be too hasty to conclude that acylated derivatives of methionine are not possible precursors of ethylene. In this respect it may be of interest to note that even methionine (externally added) is inhibitory to ethylene production in certain systems (43, 48, 82). However, if methionine synthesis is blocked by the addition of lysine and threonine, the ethylene production is considerably reduced (48).

5. Effect of Auxin on Ethylene Production

The synthesis of ethylene in plants is controlled by various physiological conditions. Not all parts of a plant are found to produce the same amount of ethylene. Young leaves of vegetative plants, for example, produce more than old (130). In fruits, however, the situation is reversed. Immature fruits produce less ethylene than ripening fruits. Analysis of gas samples withdrawn from the central cavity of melons has shown an internal ethylene concentration of 0.04 ppm at ten days after pollination. It rises to 10-70 ppm just prior to the climacteric — an increase of 1000 fold (98). Flowers also produce measurable quantities of ethylene (135).

There appears to be a close relationship between ethylene production in a tissue and its auxin content. Zimmerman and Wilcoxon (187) first discovered that auxin increased ethylene production. Kinetin has been found to increase this auxin effect greatly (55). Burg and Clagett (28) reported no conversion of methionine to ethylene unless the pea stem tissue sections were first treated with indoleacetic acid (IAA). It was suggested that auxin might stimulate an enzyme system involved in ethylene synthesis (4). Kang _et al_. (68) and Sakai and Imaseki (154) proposed that auxin induced production of a short-lived RNA that was involved in snythesizing an extremely labile protein controlling the rate of ethylene production. Such a system required an induction period. This auxin-stimulated ethylene production could be inhibited by cycloheximide (83, 168). However, there are reports that ethylene production is stimulated immediately after the application of auxin in pea stems (36) and in mung bean stem tissue (154). Steen and Chadwick (168) found that at low concentrations of IAA (1 μM) the stimulation of ethylene production did not involve any protein synthesis and therefore cycloheximide did not have an effect at this concentration of IAA. At high concentrations of IAA (100 μM) an induction period preceded the stimulatory action and was inhibited by cycloheximide. The authors suggested that there are two systems producing ethylene — a stable basal system insensitive to IAA and cycloheximide and a labile, auxin-induced system that normally accounts for about one-half the ethylene production. The latter responded immediately to low concentrations of applied IAA, ethylene production increasing several fold in rate without a significant lag period. At higher IAA concentrations the ethylene production was induced after a considerable lag. Thus the ineffectiveness observed by Burg (26) of cycloheximide in prevention of ethylene production in apple tissue might be because of low concentrations of auxin in the fruit.

The mechanism by which auxin stimulates ethylene production is not clearly understood. The situation is further complicated by our lack of understanding of the pathways of ethylene biogenesis. Recently Kang _et al._ (69) observed that ethylene production by pea stems after the application of IAA closely followed the levels of free unconjugated IAA in tissue. This is based on the observation that with a synthetic auxin like 2,4-D, which is not subject to detoxification by forming conjugated compounds (mainly the aspartate conjugate), the production of ethylene remains high.

It has been shown that the oxygen uptake caused by oxidative phosphorylation can be nearly doubled by the application of 1×10^{-10} M IAA to the mitochondria isolated from cotyledons of corn (155). In this respect it is interesting to note that indoleacetic acid, which is known to stimulate ethylene synthesis (28, 55) binds to tRNA (18, 72, 75). One of the species of tRNA to which IAA binds is tRNA^{met}, others being tRNA^{Gly}, tRNA^{val}, tRNA^{ala} and tRNA^{glu} (81). There is no indication that IAA binds to $\text{tRNA}^{\text{f-met}}$. Armstrong (9) has suggested that auxin in higher plant cells might supplement or replace N-formylmethionine as a signal for peptide chain initiation. N-formylmethionine has been shown in model systems to be a better substrate for ethylene production than methionine itself. The stimulatory effect of auxin on ethylene biosynthesis, therefore, may partly be a result of its taking over the role of formylmethionine in protein chain initiation and making the latter (probably in the form of f-met-tRNA) available as a substrate for ethylene production.

6. Inhibitors of Ethylene Biosynthesis

A number of workers (35, 70, 158) have shown that oxidative metabolism is required for ethylene formation, and blocking ATP formation by 2,4-dinitrophenol or other compounds results in the inhibition of ethylene production. Copper binding agents such as cuprizone (biscyclonexanoneoxalyldihydrazone) and bathocuproine sulphonic acid (2,9 dimethyl-4,7-diphenyl phenanthroline 1,10-disulphonic acid) inhibited ethylene production strongly at a concentration of 10^{-4} M (112). Inhibitors of the terminal oxidase of the mitochondrial electron transport chain, like cyanide and azide, also seem to decrease ethylene production (112, 113, 158). However, Burg and Thimman (36) found that cyanide did not inhibit ethylene production in apple tissue. Other inhibitors of cell metabolism like fluoride, arsenite, iodoacetate and malonate were also found to be inhibitory to ethylene production (35, 158). Aldehyde trapping agents like dimedone (5,5-dimethyl 1,3-cyclohexanedione) were reported to inhibit ethylene production completely in fungi without affecting growth (2). Another aldehyde fixing agent, sodium bisulphite, was found to have a similar effect on ethylene production by apple tissue (35).

Owens _et al_. (132) reported that a phytotoxin produced by certain strains of soyabean root nodule bacterium _Rhizobium japonicum_ inhibited ethylene biosynthesis in sorghum seedlings and in senescent apple tissue by blocking the conversion of methionine to ethylene. This toxin was named rhizobitoxine and its chemical structure has been elucidated: [2-amino-4-(2'-amino-3'-hydroxypropoxy)-trans-3-butenoic acid] (131). It is known that rhizobitoxine inhibits the growth of Salmonella typhimurium by inhibiting β-cystathionase, an enzyme in the methionine biosynthetic pathway (133). Similar inhibitory effects of rhizobitoxine on spinach and corn seedling β-cystathionase has been reported (59, 60) and the mechanism of inactivation of the enzyme, it is suggested, involves formation of a bond between rhizobitoxine and pyridoxal phosphate. Owens et al. (132) proposed that inhibition of ethylene production from methionine was by a similar mechanims probably involving pyridoxal phosphate as in the case of β-cystathionase. There is evidence for the existence of a pyridoxal dependent enzyme in the conversion of methionine to ethylene. Thus L-canaline, which is known to be an inhibitor of pyridoxal enzymes (142) also inhibits ethylene production from labelled methionine in apple tissue (184).

These observations led to further investigations into the nature of the inhibition of ethylene biosynthesis and other structurally related inhibitors. Thus, L-2-amino-4-(2'-aminoethoxy)-trans-3-butenoic acid were found to equally inhibit ethylene production in fruit and other plant tissues (84). These alkyl substituted enol ether amino acids were believed to produce their effect by antagonizing the utilization of methionine in the pathway for ethylene biosynthesis. However, inhibitors of methionine adenosyl transferase reaction (ATP:L-methionine S-adenosyl transferase (EC 2·4·2·13)), like DL-2-amino-trans-4-hexenoic acid and L-2-amino-4-hexynoic acid, were without effect on ethylene biosynthesis. This observation indirectly suggests that S-adenosyl methionine is probably not an intermediate in the ethylene biosynthetic pathway as was originally proposed (28, 30).

8. Regulation of Ethylene Biosynthesis

While the use of inhibitors may provide a powerful tool in the elucidation of the mechanism of ethylene biosynthesis, it does not tell us anything about the mode of regulation of ethylene production in plants. Are there natural inhibitors the effect of which can vary with changing physiological conditions, present in tissues? It is known that certain monohydroxy and dihydroxy phenols are inhibitory to ethylene production (10). Mapson and Wardale (112) reported that caffeic acid and ferulic acid were powerful inhibitors of ethylene production in cauliflower extracts, while cinnamic acid, p-coumaric acid and phenol had little or no effect. It is known that wounding a tissue increases the phenol oxidase activity, and at the same time increases ethylene production (140). Is the increased ethylene production an effect of the removal of the inhibitory effect phenolic compounds on ethylene production? No information correlating these two aspects is available. Also it is difficult to explain the increased ethylene production associated with different kinds of mechanical and chemical stress other than wounding, in terms of this model.

The existence of specific regulators or inhibitors of ethylene biogenesis is also suggested by the observation of Meigh _et al_. (116) that ethylene production is partially inhibited in apples attached to the tree. The nature and the mode of action of such regulators are not known.

It has been known for some time that ethylene is an essential factor for fruit ripening (32). Controlled atmosphere (high CO_2, low O_2) storage of fruits delays their ripening (32, 147) and this is thought to be a result of the antagonistic effect of carbon dioxide on the physiological effects of ethylene (2). Attempts have been made to explain this effect by the competitive action of carbon dioxide at the "ethylene receptor site" possibly involving a metal ion (30, 32). But controlled atmosphere storage conditions also decrease the amount of ethylene produced by fruits (122). Meigh (115) has shown that a high carbon dioxide concentration retards ethylene production by stored apples. A similar phenomenon was observed in avacado, banana and mango (32). Thus, while the "competetive action theory" might explain the inhibitory action of CO_2 on ethylene effects, it does not tell us anything about the mechanism by which CO_2 inhibits ethylene production under controlled atmosphere storage. Any theory explaining the inhibitory effect of carbon dioxide on fruit ripening should take into account its effect on both ethylene action and production.

The ripening process results in a decrease in the organic acid contents, especially that of malic acid, of fruits (20, 122, 147, 182). This decrease of organic acids was much smaller under controlled atmosphere storage conditions. Do organic acids have an inhibitory effect on ethylene production? And does carbon dioxide help to maintain the organic acid concentration of fruits at a high level? Studies made by Rhodes _et al_. (147) and Murata and Minamide (122) suggest that the malic acid content of apples stored under controlled atmosphere storage conditions does not decrease to any great extent. Carbon dioxide is fixed under these storage conditions by apples, and most of the label of $^{14}CO_2$ appears in malic acid (122). This carbon dioxide fixation could be catalyzed by malic enzyme and/or phosphoenol pyruvate (PEP) carboxylase. Reactions catalyzed by these enzymes are given below:

malic enzyme [L-malate:NADP oxidoreductase (EC 1·1·1·40)]

$$\text{Pyruvate} + CO_2 + \text{NADPH} + H^+ \overset{Mn^{2+}}{\rightleftharpoons} \text{L-malate} + \text{NADP}$$

PEP-carboxylase (EC 4·1·1·31)

$$\text{PEP} + CO_2 + H_2O \overset{Mg^{2+}}{\rightleftharpoons} \text{oxaloacetate} + \text{Pi}$$

Rhodes et al. (147) have shown that during ripening of apples the malate decarboxylation system is stimulated and externally added ethylene hastens this stimulation. The authors suggested that malic enzyme, pyruvate decarboxylase and alcohol dehydrogenase act in concerted manner in the decarboxylation of malate. Apart from the probable removal of the inhibitory effect on ethylene synthesis, decarboxylation of malate seems to be extremely important from a bioxynthetic standpoint. The reaction catalyzed by malic enzyme provides NADPH needed for many biosynthetic reactions and cannot be obtained in adequate amounts from the pentose phosphate pathway, since the activity of this pathway is reduced in ripening fruits (63) and in the presence of externally added ethylene (154).

The reaction catalyzed by malic enzyme is readily reversible and is one of the reactions that plants utilize in the dark fixation of CO_2. Fixation of CO_2 into malate does occur in apples under controlled atmosphere storage conditions, and also under these conditions ethylene production from labelled methionine is decreased (122). Whether malate exerts an inhibitory effect on ethylene production is not yet answered directly.

It is interesting to note that to offset the physiological effects of ethylene, the concentrations of CO_2 required is a million times that of the olefin. This high ratio of CO_2 to ethylene does not fit very well into a model where CO_2 binds to the ethylene receptor site, and thus removes its physiological effects. On the other hand, if the role of the CO_2 is to shift the equilibrium of the reaction catalyzed by malic enzyme towards the synthesis of malate, which in turn exerts an inhibitory effect on ethylene production, one can easily visualize the requirement for a fairly high concentration of carbon dioxide.

9. Ethylene Production by Microorganisms

It is fairly well established that ethylene is a normal metabolite of several fungi (65, 66). The high rate of ethylene production and the ease of introducing proposed intermediates offered distinct advantages to investigators working with fungi. Thus ethylene production by Penicillium has been studied intensively. In spite of considerable effort by a large number of workers, the biochemistry of ethylene formation in this organism remains unknown. Various precursors of ethylene in this organism have been proposed. Among them are glucose (137), alanine (136), glycine (179), aspartic acid (136), glutamic acid (136), methionine (68), serine (167), propionate (68), ethanol (136), acetate (68), citrate (71), fumarate (68), malate (136), pyruvate (58), and succinate (68). Most of these workers used an excessively long incubation period in their feeding experiments. For example, Jacobsen and Wang (68) collected ethylene for 24 hours after the addition of various labelled carbon compounds, and this long duration of time allowed the label to distribute in various compounds.

Even though methionine is fairly well accepted as a precursor of ethylene in higher plants, doubts have been expressed whether this compound serves as a source of ethylene in Penicillium and it was suggested that the ethylene carbon skeleton was derived from internal carbon atoms of dicarboxylic acids of the Krebs cycle (68). Kerting et al (71) showed that ethylene was produced from isocitrate, but kinetic studies made by Chou and Yang (40) showed that the rate of incorporation of radioactivity into ethylene from ^{14}C labeled 2-oxoglutarate and L-glutamate was faster than that from labeled isocitrate. On this basis these authors concluded that L-glutamate was a more immediate precursor of ethylene than isocitrate.

Methionine does not seem to be a precursor of ethylene in Penicillium. This observation was further supported by the finding that rhizobitoxine, which normally inhibited conversion of methionine to ethylene was without any effect on ethylene production in this organism (132).

It has been shown that some other microorganisms do utilize methionine as a precursor of ethylene. Thus Lund and Mapson (92) found that the bacterium Erwina carotovora converted methionine to ethylene. Ceratocystis fimbriata, an organism that infests sweet potato, also is known to produce ethylene from methionine (70).

Lynch (96) isolated a number of soil microorganisms that were capable of producing ethylene from methionine. Two of the isolates that produced maximum amounts of ethylene were yeasts. Lynch and Harper (93) reported that the soil fungus Mucor hiemalis produced ethylene from methionine, but glucose was also required for maximal ethylene production. In addition, they found that DL-ethionine also could serve as substrate for ethylene formation, but other compounds like β-alanine, glucose, acetate, citrate, pyruvate, ethanol, noreucine, lysine and serine did not have any stimulatory effect on ethylene production.

B. PHYSIOLOGICAL AND BIOCHEMICAL EFFECTS OF ETHYLENE

1. Auxin and Physiological Effects of Ethylene

Auxin is known to regulate the synthesis of many kinds of enzymes, e.g. cellulase (101), hemicellulase and β-1,3 glucanase (176) and peroxidase (56). The syntheses of these enzymes, among others, are also affected by ethylene (7, 144, 148). Thus some of the normally observed effects of auxin may actually be attributable to ethylene.

It appears that the physiological effects of ethylene are lowest when the auxin content of the tissue is the highest. Young leaves are not as susceptible to ethylene as old ones, even though the former contain relatively larger concentrations of auxin and produce greater amounts of ethylene than the latter (130). Frankel and Dyck (52) observed that indolacetic acid and 2,4-D prevented the climacteric rise in respiration, softening and degreening of Bartlett pears, even though ethylene production was stimulated. They proposed that endogenous auxin in fruit represented a resistance factor in ripening and must be inactivated before ripening could occur.

All physiological effects of ethylene are not suppressed in the presence of auxin. Thus auxin-induced ethylene production plays a role in leaf abscission (5), inhibition of flowering in xanthium (6), inhibition of bud growth, fading of orchid flowers (27), and isocoumarin formation in carrots (37). Also Apelbaum and Burg (12) have shown that application of 0.1 mM 2,4-D reverses the growth inhibition caused by ethylene, but stimulates formation of sufficient amounts of ethylene to induce a swelling response.

The major cause of the overall growth inhibition in the presence of ethylene is the cessation or retardation of the mitotic process in the meristems of root, shoot and auxiliary buds (12, 13). Ethylene is known to inhibit DNA systhesis by decreasing the activity of DNA polymerase (11).

However, Klyne (74) has shown that DNA levels of tulip bulbs treated with ethylene increases after a 2-day exposure period. On prolonged exposure, the DNA content, however, decreases.

2. Effect of Ethylene on Respiration

Exogenous ethylene causes a rise in respiration when applied to climacteric or non-climacteric fruits (21, 47) and vegetative tissues (46, 154). The ethylene-induced increase in respiration of non-climacteric fruits and vegetative tissues return to the basal level on removal of the applied ethylene (47, 146). This stimulation of respiration can be repeated in the same fruits several times (21, 146). Recently it has been shown that propylene causes a rise in respiration rate in non-climacteric fruits with no change in ethylene production (100). Exogenous ethylene or propylene treatment does not stimulate ethylene production in rin tomato mutant, but does stimulate CO_2 production (64). The CO_2 production persists only in the presence of exogenous olefins.

Exogenously applied ethylene stimulates respiration and ripening in mature, unripe climateric fruits (21). Once stimulated by exogenous ethylene or by their own ethylene, climacteric fruits produce ethylene 'autocatalytically'. Propylene treatment of these fruits stimulates both ethylene production and respiration (100).

a. Mechanism of stimulation of respiration by ethylene

Crane (42) has suggested that the increased respiration rate could result from increased mitochondrial permeability and enzyme activity caused by swelling of the mitochondria. Lyons and Pratt (97) and Olson and Spencer (128, 129) found that ethylene increased the rate of spontaneous swelling of mitochondria isolated from different sources. It has been suggested that the swelling of mitochondria results from an increased adenosine triphosphatase (ATPase) activity, and the increased availability of ADP and Pi increases the respiration (129). Phillips (138) showed that treating pea mitochondria with ethylene stimulated ATPase activity, even though purified ATPase did not respond to ethylene.

Swelling of mitochondria and increasing respiration are not effects specific to ethylene. It has been shown that other hydrocarbons like propene, butene, ethane, propane (78, 117) and acetylene (117) could also bring about mitochondrial swelling and an increase in the rate of respiration.

It is well known that plant tissues produce abundant ethylene when damaged (140). Subsequently, the respiration of the tissue increases. It has been shown that potato slices, like many other injured plant tissues, produce significantly increased amounts of ethylene for 12 hours after cutting and respiration continues to increase even after that (99). Reid and Pratt (146) postulated that the increase in respiration might be induced by endogenous ethylene.

The induced respiration has some qualitative differences from that of fresh tissue. It seems clear now that one of the changes is an increased participation of a cyanide resistant respiratory pathway (62). Cyanide at low concentrations can evoke the same kinds of response in fruits (22) and in potato tubers (160) as ethylene. Solomos and Laties (161) proposed that ethylene diverts the normal flow of electrons to the cyanide resistant respiratory chain. This pathway has only one phosphorylation site (17), and to produce an equal amount of ATP as in normal respiratory chain, three times more electrons have to pass through this branched pathway. Thus increased flow of electrons (increased respiration) in the presence of ethylene actually compensates for a fewer number of phosphorylation sites.

3. Effects of Ethylene on Membrane Permeability

The possibility that ethylene regulates physiological phenomena by virtue of some effect on the permeability of membranes has been tested by several workers. Sacher and Salminen (152) have shown that ethylene does not have any effect on membrane permeability of avocado, banana, bean and rhoeo. Abeles (2) concludes that changes in the characteristics of membranes are a result of ripening rather than a cause. Nevertheless, the idea that ethylene could affect the membrane permeability has not been abandoned. Solomos and Laties (162) suggested that ethylene could act as membrane "perturber" and the subtle permeability changes thus brought about might have profound physiological effects.

Irvine and Osborne (67) reported that the incorporation of 1-^{14}C-glycerol into phospholipids of etiolated pea stems was decreased by 50% after 2-3 hours treatment with 10 ppm ethylene. The rate of incorporation remained at this value as long as ethylene was applied. The authors claimed that the results described in this experiment were the most rapid biochemical effects of ethylene in plants reported so far. The 50% decrease in 1-^{14}C-glycerol incorporation into phospholipids after 2-3 hours of application of ethylene and a similar response induced by endogenous wound ethylene implicates membranes and their metabolism as central to the mediation of ethylene response.

4. Interaction of Carbon Dioxide and Ethylene

Carbon dioxide has long been recognized as having an effect opposite to ethylene. Ethylene accelerates, and carbon dioxide delays leaf abscission and fruit ripening. The effective concentration of ethylene that is required to cause a physiological change is dependent on the concentration of CO_2. Thus when ethylene concentration is less than 1 ppm its effectiveness in affecting pea straight growth in considerably diminished with increasing concentration of CO_2 (31).

Certain physiological changes are brought about only if both CO_2 and ethylene are present. The thermodormancy of germinating lettuce seeds can be overcome by treating the seeds with a mixture of CO_2 and ethylene (127). Either gas alone is ineffective. Also inhibition of germination of lettuce seeds caused by 0.2 M NaCl or 0.35 M mannitol can be overcome by a mixture of ethylene and CO_2 (159). Ku et al. (77) found that the growth rate of rice coleoptiles was increased by low concentrations of ethylene and this response was enhanced by CO_2. Ethylene and carbon dioxide also have been shown to have a synergistic effect in terminating dormancy of potato tubers (145).

Malhotra and Spencer (105) have shown that a mixture of ethylene and carbon dioxide stimulated the activity of ($Na^+ + K^+$) stimulated adenosine triphosphatase, isolated from pea cotyledon mitochondria. Ethylene alone was ineffective and carbon dioxide alone inhibited the hydrolysis of ATP catalyzed by this enzyme. With increasing purification of the enzyme the effectiveness of gas mixtures in stimulating the enzyme decreased and this was thought to be a result of the removal of phospholipids (membrane) during the purification. This work has shown a direct action of ethylene on an enzyme [mitochondrial ($Na^+ + K^+$) - stimulated ATPase], its dependence on the presence of certain levels of CO_2 and the augmentation of the ethylene-CO_2 effects by the presence of phospholipids.

5. Effects of Ethylene on Yeast

Even though yeast is known to produce ethylene (65), no serious attention has been paid to study the effects of ethylene on yeast metabolism. Abeles in his review (2) states that ethylene has no effect on yeast physiology.

Nord and Franke (126) reported that ethylene protected zymase solutions stored at -10° and the gas treated solutions maintained their full activity for 65 days. Recently, Fuchs and Gertman (53, 54) demonstrated that incubating yeast alcohol dehydrogenase in an ethylene atmosphere stabilizes the enzyme activity. The stabilizing effect of acetylene and ethane was similar to that of ethylene, but the latter was quantitatively a more potent agent.

Yeast cells treated with ethylene, fermented glucose at a faster initial rate than untreated cells (126), and it was suggested that ethylene increased the membrane permeability. Nord and Weichherz (125) reported that both acetylene and ethylene increased the permeability of yeast cells, the former being far more effective. Treatment of yeast maceration juice with acetylene or ethylene resulted in an increased surface tension and decreased viscosity. The authors concluded that this phenomenon represented a physical adsorption of ethylene on the lipophilic colloidal particles and that ethylene exerted an inhibitory, as well as protective, action on enzymes. A similar observation was made by the same authors with germinating barley (124).

Shaw (156) observed that treating yeast with ethylene increased the rate of inversion of sucrose, and also resulted in an increase in the amount of invertase per unit weight of cells. He was unable to demonstrate any increase in the permeability of cells. He concluded from this work that the mechanism of the action of ethylene was not mediated through increased permeability, but by alteration of the cell metabolism so as to bring an increased production of enzymes.

III. MATERIALS AND METHODS

A. MATERIALS

1. Chemicals

Routine chemicals of reagent grade (meeting A.C.S. Specifications) were purchased from Fisher Scientific Co., Canadian Laboratory Supplies, and Eastman-Kodak Co. Common biochemicals were obtained either from P.L. Biochemicals or Sigma Chemical Co. Glucose-3,4-^{14}C, L-methionine-U-^{14}C, 3-O-methyl glucose-U-^{14}C and Aquasol were supplied by New England Nuclear Corp. L-methionine, D-methionine, N-formylmethionine, L-ethionine, L-alanine, β-alanine, L-cysteine, methional, potassium pyruvate, 3-O-methyl glucose and all enzymes used in this study were obtained from Sigma Chemical Co. Ethylene and ethane were purchased from Linde.

2. Yeasts

Two haploid strains of Saccharomyces cerevisiae were used in this study. The Strain X-2180-1B is a wild type while the other (G1332) is a mutant that requires both adenine and methionine for growth. Both of these strains were supplied by Dr. VonBorstel of the Genetics Department of the University of Alberta. Respiration deficient mutants were isolated from the wild strain X-2180-1B.

B. METHODS

1. Maintenance of the Yeasts

The yeasts were maintained at 0-4° by subculturing every 3 months on glucose-yeast extract agar of the following composition: glucose 2.0%, yeast extract (Difco) 0.5%, $(NH_4)_2HPO_4$ 0.6%, $MgSO_4 7H_2O$ 0.2% and agar 2.0%. The pH of the medium was adjusted to 5.0. The yeasts were grown on slants for 48 hours at 27° before transferring them to refrigerator (0-4°).

2. Cultivation of Yeasts for the Study of Ethylene Biosynthesis and the Biochemical Effects of Ethylene

The wild strain Saccharomyces cerevisiae (X-2180-1B) was used in most of the studies. It was grown at 27° in lactate or glucose medium with continuous shaking. The composition of the media are given below.

a. Lactate medium

Lactic acid (85%)	16.0 g
$(NH_4)_2HPO_4$	6.0 g
$MgSO_4 \cdot 7H_2O$	2.0 g
Yeast extract (Difco)	5.0 g
Distilled water to	1000 ml

The pH of the medium was adjusted to 5.0 with potassium hybroxide.

b. Glucose medium

D-glucose	20.0 g
K_2HPO_4	3.0 g
$(NH_4)_2HPO_4$	6.0 g
$MgSO_4 \cdot 7H_2O$	2.0 g
Yeast extract (Difco)	5.0 g
Distilled water to	1000 ml

The pH of the medium was adjusted to 5.0 with ortho-phosphoric acid.

The adenine- and methionine-requiring mutant yeast (Saccharomyces cerevisiae G1332) was grown in a liquid medium of the following composition:

D-glucose	20.0 g
Yeast nitrogen base without amino acids (Difco)	6.8 g

L-methionine	0.2 g
Adenine	0.2 g
Distilled water to	1000 ml

The pH was adjusted to 5.0 with phosphoric acid.

3. Isolation of Respiration Deficient Mutants

The wild yeast (Saccharomyces cerevisiae X-2180-1B) was plated on a medium that contained 3% glycerol, 0.1% glucose, 0.6% $(NH_4)_2HPO_4$, 0.2% $MgSO_4$, 0.1% yeast extract and 2% agar. The plates were incubated at 27° for 72 hours. The petite colonies formed were transferred to a growth medium that contained 2% glucose. Only those colonies that grew in glucose medium, but failed to grow in lactate medium were considered as respiration deficient. They were further tested for respiratory activity (described in a separate section), and only those isolates that did not have measurable respiratory activity were used for further study.

4. Starvation of the Yeast

Saccharomyces cerevisiae (X-2180-1B) was grown in lactate medium at 24-26° for hours. The cells were harvested by centrifugation, washed twice with distilled water, and suspended in 0.1 M potassium phosphate buffer (pH 5.0). Air or 100 ppm ethylene in air was bubbled through the suspension at the rate of 100 ml per minute for different lengths of time.

5. Anaerobic Cultivation of Yeast

Saccharomyces cerevisiae (X-2180-1B) was grown at 27° in the glucose medium, which in addition to the ingredients mentioned previously, contained 0.01% ergosterol and 0.01% Tween-80. Anoxic conditions were maintained by bubbling sterile nitrogen (sterilized by filtering through sterile cotton) through the gorwth medium at the rate of 100 ml per min.

6. Cultivation of Yeast in the Presence of Applied Ethylene

Saccharomyces cerevisiae (X-2180-1B) was grown in lactate or glucose medium at 27° on a reciprocal shaker (60 strokes per min). Filtered air or 100 ppm ethylene in air was bubbled through the culture continuously at the rate of 100 ml per minute. The gases were filtered by passing through sterile cotton before admitting them into the culture.

7. Collection of Ethylene

The method used was essentially the same as described by Ghooprasert (57). A continuous stream of compressed air or helium was freed of ethylene and ethane by passing it through mercuric perchlorate adsorbed on silica gel (80-200 mesh) in a U-tube that was placed in an ice bath, followed by two silica gel-containing U-tubes placed in dry ice-acetone baths. This "purified sweeping gas" flowed through flow meters into reaction flasks that contained suitable volumes of material under study. The flow rate (30 ml per minute) of the sweeping gas was accurately regulated by means of needle valves. The temperature of the reaction flasks was maintained at 25° in a shaker-water bath. The gas flowing out of the reaction vessel carried ethylene and other volatiles evolved by the test material. The gas was passed through a U-tube containing Drierite to remove moisture, a U-tube containing Lithosorb to remove CO_2 and an empty U-tube dipped in a dry ice-acetone bath to condense a number of other volatiles (but not ethylene). Ethylene coming out of this series of tubes was quantitatively adsorbed on 1.0 g of silica gel contained in a U-tube placed in a dry ice-acetone bath. (NOTE: Prior to use all U-tubes containing silica gel were heated in a boiling water bath with a stream of helium flowing through the tube. This process removed any trace of ethylene adhering to the silica gel.) After the collection, the U-tubes were sealed by short pieces of white rubber tubing and kept in a dry ice-acetone bath until ethylene was determined. Determination of ethylene was usually completed within one hour of collection.

8. Determination of Ethylene by Gas Chromatography

Ethylene adsorbed on silica gel in U-tubes was determined by a gas chromatograph with a flame ionization detector. For this purpose the U-tube (still in the dry ice-acetone bath) was attached to the gas outlet side of a two-way valve inserted into the helium line of a Perkin-Elmer model 811 flame ionization gas chromatograph. The other end of the tube was left open to the atmosphere. The valve was opened and the U-tube was flushed with helium at the rate of 40 ml per minute for 50 seconds. After this the open end of the U-tube was attached to the line and the flushing continued for another 10 seconds. This flushing procedure was found to be necessary to prevent the flame being put out when the sample was introduced into the column by opening the valve.

After flushing, the dry ice-acetone bath was removed and the U-tube heated in a 60° water bath for 3 minutes to release adsorbed gases from silica gel. The volatile samples were admitted to the column by opening the valve and flushing the U-tube with carrier gas. The column was a 46 cm x 0.6 cm stainless steel tube packed with 80-100 mesh activated alumina. The column was operated at 45° and the detector temperature was 110°. Under these conditions four peaks were obtained. The identities of first and fourth peaks are not known. The second and third peaks have been identified as ethane and ethylene, respectively, by co-chromatography using authentic samples. Calibration curves with known amounts of standard ethylene were prepared. Peak heights and peak areas were found to be proportional to the amounts of ethylene used.

9. Determination of the Radioactivity of Ethylene Produced From L-Methionine-U-^{14}C

Ethylene was collected as outlined in section 7 above. Part of the ethylene coming out of the gas chromatograph column was absorbed in mercuric acetate solution. The mercuric acetate was prepared as described by Abeles (1). Twenty grams of mercuric acetate was dissolved in 500 ml of methanol plus 1 ml of glacial acetic acid. A flow splitter was utilized to direct 2/3 of the sample to 3 ml of mercuric acetate solution and the remaining 1/3 to the gas chromatograph flame. After the ethylene peak had emerged, 1 ml of the sample was transferred to a scintillation vial, 10 ml of Aquasol was added, and the radioactivity determined in a Nuclear Chicago Scintillation counter.

10. Measurement of Respiration

a. Warburg method

Saccharomyces cerevisiae (X-2180-1B) was grown in lactate medium in the presence of air or 100 ppm ethylene in air as described earlier. The cells were harvested by centrifugation, washed twice with distilled water, and suspended in 0.1 M potassium phosphate buffer (pH 5.0). The rate of oxygen uptake was determined by the direct Warburg method using 0.5 ml yeast suspension and 2.5 ml of potassium phosphate buffer containing 15 mg of glucose (total volume 3.0 ml).

b. Biological oxygen monitor method

This method was used to measure the rate of respiration of the yeast that was starved in presence of air or 100 ppm ethylene in air. The rate of oxygen uptake was measured in a biological oxygen monitor (Yellow Spring Instruments) with a Clarke oxygen electrode. The reaction mixture contained 2.8 or 2.9 ml of 0.1 M potassium phosphate buffer (pH 5.0) and 0.1 or 0.2 ml of yeast suspension. A 100% saturation was calcualted to be equivalent to 260 nmoles of oxygen per ml.

11. Determination of Growth Rate

The growth rate was followed by measuring the absorbance at 650 nm in a Bausch and Lamb spectronic-20 photoelectric colorimeter.

12. Determination of Dry Weight

A suitable volume of cell suspension was filtered through a pre-weighed Millipore filter (47 mm diameter and 0.8 pore size), washed 3 times with distilled water and dried in an over at 60° under vacum for 18-24 hours. The dried samples were cooled in a desiccator and then weighed.

13. Determination of Ethanol

Suitable volumes (0.4 ml to 4 ml) of yeast culture were chilled in ice, and the cells removed by centrifugation. The supernatant layer was diluted with double distilled water and the ethanol content determined enzymatically by the method described by Bonnischsen (23). The assay medium contained 3 ml of pyrophosphate--semicarbazide buffer pH 8.6, 0.1-0.2 ml supernatant layer and 0.5 mg NAD. The reaction was started by adding 8-10 units of alcohol dehydrogenase. The absorbance was measured at 340 nm in a Beckman DU-spectrophotometer 90 minutes after starting the reaction. A standard was run every time with a known concentration of pure ethanol.

14. Determination of Ethanol Production by Yeast in the Presence of Metabolic CO_2 and Applied Ethylene

Saccharomyces cerevisiae (X-2180-1B) was grown in lactate medium to the mid-log phase (16-18 hours). Cells were harvested by centrifugation, washed twice with distilled water, and suspended in 0.1 M potassium phosphate buffer (pH 5.0) containing 5% glucose. The suspension was distributed in 20 ml quantities into twelve 50 ml-flat bottom flasks fitted with tight fitting rubber stoppers that had 2.5 ml plastic syringes fixed on them. This allowed sampling of liquid from the flasks without opening them. The CO_2 produced during fermentation of glucose was trapped in some flasks by 1 ml of 20% KOH placed inside the flask in a scintillation vial. Fluted filter paper, which dipped into alkali and projected above the neck of the vial, gave maximum surface area for the adsorption of CO_2.

Each flask was flushed for 5 min with appropriate concentration of ethylene or air at the rate of 200 ml per minute, and then tightly closed. The flasks were incubated at 24-26° on a reciprocal shaker (60 strokes/minute). At regular intervals samples (0.4-0.5 ml) were withdrawn and the ethanol content in them determined enzymatically.

15. Determination Glucose Uptake

Log-phase cells obtained by growing S. cerevisiae (X-2180-1B) in lactate medium were washed and suspended in 0.1 M potassium phosphate buffer (pH 5.0). Suspensions were aerated for 10 minutes with air or 100 ppm ethylene in air, at the rate of 100 ml per minute. Glucose was added in the dry form to the suspension to give a final concentration of 0.5% and the aeration with the particular gas continued. At definite time intervals aliquots of the suspension were withdrawn, cells separated by Millipore membrane filters (0.8 μ) and the amount of glucose remaining in the filtrate determined with glucose oxidase and peroxidase as described by Bergmeyer and Bernt (19).

16. Determination of 3-O-Methyl Glucose-U-^{14}C Uptake

Experimental details were the same as in the case of glucose uptake except different concentrations (1 mM to 50 mM) of 3-O-methyl glucose-U-^{14}C (4,990,000 dpm or 2.24 μci/mmole) were used. After 20 minutes 2 ml portions of the suspensions were removed, and filtered through a Millipore membrane filter (0.8 μ). The cells on the filter were washed three times with 5 ml portions of 0.1 M potassium phosphate buffer (pH 5.0) containing 50 mM unlabelled 3-O methyl glucose. The filter was transferred to a scintillation vial, 10 ml of Aquasol added and the radioactivity determined in a Nuclear Chicago scintillation counter (model Unilux II).

17. Determination of $^{14}CO_2$ Production by Yeast from Glucose-3,4-^{14}C

Washed yeast cells were suspended in 15 ml of 0.1 M potassium phosphate buffer (pH 5.0) in a radiorespirometric flask described by Wang (178). The side arm contained 4.2 mmoles of glucose-3,4-^{14}C in a volume of 1.5 ml (sp. activity 104,760 dpm/mmole). Air or 100 ppm ethylene in air was bubbled through the suspension at the rate of 50 ml per minute. After 10 minutes the glucose solution was tipped in. Carbon dioxide evolved was absorbed by passing the gas coming out of the respirometric flask through 10 ml of methanol:ethanolamine mixture (70:30). After 30 minutes, the carbon dioxide absorbing reagent was removed, and it was replaced by 10 ml of the fresh reagent. This was repeated every 30 minutes. Radioactivity of the $^{14}CO_2$ absorbed in the methanol: ethanolamine mixture was determined by transferring 5 ml of the mixture to scintillation vial, adding 10 ml Aquasol and counting in a Nuclear Chicago scintillation counter (model Unilux II).

18. Preparation of Yeast Extract and Assay of Enzymes

The method described by Maitra and Lobo (103) for the preparation of yeast extract and assay of enzymes was used with some modifications. At a suitable growth stage a 20-30 ml portion of growing culture was chilled with ice, centrifuged, and washed once with 150 mM KCl. The cell pellet was suspended in 2 ml of the medium (2 mM 2-mercaptoethanol, 2 mM EDTA, 50 mM potassium phosphate buffer pH 7.4). After the addition of three drops of toluene, the suspension was incubated for 30 minutes in a 37° water bath. After a preliminary clarification at low speed the suspension was finally centrifuged at 15,000 x g for 10 minutes. The supernatant layer thus obtained served as the enzyme solution in all of the experiments.

All enzymes were assayed by coupling the particular step to the appropriate NAD- or NADP-linked reaction with the use of commercially available enzymes as coupling enzymes. The rate of production or disappearance of reduced pyridine nucleotides was followed continuously on a Turner model 111 fluorometer fitted with a high sensitivity door. The fluorescence change was recorded with a 10 mV potentiometric Beckman recorder. One full scale deflection of the recorder corresponded to a span of 4 to 6 nmoles of NADH or NADPH in a total volume of 4 ml at 25°. The concentration of the test enzyme was chosen so as to require 5-10 minutes to traverse the full scale. That the rate was first order with respect to the amount of enzyme solution was ensured for at least 2- and generally 3-fold amounts of the aliquots. In most cases, enzymes were diluted in cold phosphate buffer containing EDTA and mercaptoethanol. With the exception of alcohol dehydrogenase all other enzymes were assayed in a buffer (pH 7.4) containing 50 mM triethanolamine (neutralized with

KOH) and 10 mM $MgCl_2$. Unless otherwise specified all reactions were started by the addition of substrate. The total volume of the assay mixture in each case was 4 ml.

The reaction mixture for hexokinase (EC 2·7·1·1) contained 10 µmoles of glucose, 100 nmoles of ATP, 100 nmoles of NADP and 0.3 units of glucose-6-phosphate dehydrogenase in 4 ml of triethanolamine buffer. For glucose-6-phosphate dehydrogenase (EC 1·1·1·49) 5 µmoles of glucose-6-phosphate, 100 nmoles of ATP and 100 nmoles of NADP were used. Gluconate-6-phosphate dehydrogenase (EC 1·1·1·44) was assayed in the presence of 5 µmoles of gluconate-6-phosphate and 100 nmoles of NADP. For the assay of glucose-6-phosphate dehydrogenase and gluconate-6-phosphate dehydrogenase the rate in the very first minute was taken as the activity in order to minimize the contribution of gluconate-6-phosphate dehydrogenase and of NADPH, respectively.

The phosphofructo kinase (EC 2·7·1·11) assay mixture consisted of 10 µmoles of glucose-6-phosphate, 2 units of phosphoglucoisomerase, 100 nmoles of NAD, 100 nmoles of ATP, 40 nmoles of ADP, 1 unit each of aldolase and α-glucerophosphate dehydrogenase, and 10 units of triose phosphate isomerase. The reaction was started with ATP.

Glyceraldehyde-3-phosphate dehydrogenase (EC 1·2·1·12) activity was measured in a mixture containing 40 nmoles of NADH, 100 nmoles of L-cysteine, 100 nmoles of 3 phosphoglycerate, 100 nmoles of ATP and 1 unit of phosphoglycerate kinase. Pyruvate kinase (EC 2·7·1·40) was determined in a system with 40 nmoles each of phosphoenol pyruvate, ADP, fructose-1,6-diphosphate and 20 nmoles of NADH and 1 unit of muscle lactate dehydrogenase.

Alcohol dehydrogenase (EC 1·1·1·1) was assayed with 50 µmoles of ethanol, and 100 mmoles of NAD in pyrophosphate-semicarbazide-glycine buffer (pH 8.8) according to the method described by Bonnischsen (23).

19. Presentation of Enzyme Activity

The enzyme activities are expressed as milliunits per mg of extract protein, thereby normalizing the results with respect to the increase in cell mass and variable extraction of protein from the cells. The more conventional method of expressing enzyme activity per unit cell mass was not followed as breakage of the cells by toluenization led to variable extraction. The protein was determined by the method of Lowry et al. (91).

20. Determination of Glucose-6-Phosphate, ATP, ADP and L-Alanine

The method described by Maitra and Estabrook (102) for the extraction of glucose-6-phosphate, ATP and ADP from yeast was used with some modifications. Yeast cells were harvested from 5-10 ml culture by filtering through a Millipore filter (0.8 µ), and were washed 5 times with cold distilled water. The filter containing the cells was transferred to 3 ml of 5 N perchloric acid. After 30 minutes standing at room temperature the cells and debris were removed by centrifugation and an aliquot of the supernatant layer was adjusted to pH 7.4 with a neutralizing buffer. The neutralizing buffer had the following composition: 0.5 M triethanolamine (adjusted to pH 7.4 with HCl) and 0.67 M KOH. The neutralized samples were stored in ice for 30 minutes to precipitate potassium perchlorate, which was then removed by centrifugation. An aliquot of the supernatant layer was used for the determination of ATP, ADP and glucose-6-phosphate by a fluorometric method described by Maitra and Estabrook (102).

L-Alanine in the extract was determined enzymatically in the presence of 1 unit of glutamate-pyruvate transaminase, 10 µmoles of NADH, 10 µmoles of 2-oxoglutarate, 0.3 units of lactate dehydrogenase in a total volume of 4 ml. The buffer used was 50 mM triethanolamine at pH 7.4. The oxidation of NADH was determined at 340 nm in a Beckman DU spectrophotometer.

21. Determination of K^+ Uptake

Saccharomyces cerevisiae (X-2180-1B) was grown in lactate medium, and the cells were harvested while they were in the log phase of growth. They were washed twice with distilled water and then starved for 24 hours at 22-24° in 0.02 M Tris-HCl buffer pH 7.4 with continuous bubbling of oxygen through the suspension. The cells were collected by filtration and suspended in 0.02 M citrate buffer (pH 4.75, adjusted with tris) containing 3% (v/v) propan-2-ol. Ten ml of the suspension was transferred to a modified double jacketed reaction vessel (Yellow Spring Instruments Biological Oxygen Monitor). The temperature of the suspension was maintained at 25° by circulating water through the monitor jacket. Potassium chloride was added to the suspension to give a final concentration of 0.4-0.5 mM K^+. After equilibration (usually about 2 minutes) K^+ uptake was initiated by bubbling the suspension with a gas mixture consisting of 25% CO_2 and 75% air of 2000 ppm ethylene in air, at the rate of 50 ml per minute. The K^+ movements were monitored with an Orion Model 92-19 liquid membrane potassium electrode connected to a Radiometer model 26 Expanded Scale pH meter. Output of the pH meter was recorded on a Texas Servo Riter recorder. Application of a compensating voltage by means of a 100 mV 'turnpot' was found to be necessary, when a wide range of potassium ion concentrations were to be tried.

IV. RESULTS AND DISCUSSION

A. PRODUCTION OF ETHYLENE BY SACCHAROMYCES CEREVISIAE

1. Ethylene Production by Saccharomyces cerevisiae Grown in Lactate Medium

Saccharomyces cerevisiae (X-2180-1B) did not produce any ethylene when lactate was the main carbon source in the growth medium. In fact, the production of ethylene by the yeast was significantly lower than that produced by uninoculated medium (blank). The results from one of the typical experiments are presented in Table 1.

Uninoculated fresh medium produced the maximum amount of ethylene. Filtrate obtained from the culture also produced more ethylene than an equal volume of culture itself. Addition of either cycloheximide or D-threo-chloramphenicol seemed to increase production of ethylene from all samples slightly.

The yeast seems to absorb ethylene from the surrounding medium. Calculation from the data presented in Table 1 showed that 6.8 nl of ethylene were absorbed by 1 g of yeast (dry weight) per hour. This adsorption was less in the presence of cyclohexamide (4.1 nl/g/hr) and more in the presence of D-threo-chloramphenicol (9.9 nl/g/hr).

The smaller production of ethylene by the yeast compared to filtrate and fresh medium does not appear to be a result of ethylene getting dissolved in the lipid fraction of yeast. If this were the case, one would expect the amount of ethylene absorbed by the yeast to be proportional to the cell mass. Chloramphenicol treated cells absorbed more ethylene than untreated cells, even though in the former there was 17% inhibition of growth. The decreased absorption of ethylene by the

TABLE 1: Production of Ethylene by Saccharomyces cerevisiae (X-2180-1B) grown in lactate medium

Sample	nℓ Ethylene per Hour		
	Control	+ Cycloheximide	+ D-threo-Chloramphenicol
Yeast culture	0.64	0.82	1.01
Filtrate	1.10	1.10	1.65
Fresh medium	1.37	1.46	1.70
Yeast culture minus Filtrate	-0.46	-0.28	-0.64

The yeast was grown in lactate medium at 27° for 18 hours and then ethylene was collected from 20 ml of culture, filtrate or uninoculated fresh medium. Twenty ml culture contained 67 mg of yeast (dry weight). Cycloheximide (120 μg/ml) or D-threo-Chloramphenicol (1.5 mg/ml) was added at zero time of ethylene collection and the collection was continued for 60 minutes.

cycloheximide treated cells, on the other hand, may actually reflect decreased growth rate. In this case a 46% inhibition of growth was observed.

2. Induction of Ethylene Production in Yeast by Glucose

Although S. cerevisiae (X-2180-1B) does not produce any ethylene when lactate serves as the main carbon source in the growth medium, its production can be induced by the addition of glucose. A typical response to the addition of glucose in shown in Figure 1.

The yeast absorbed ethylene from the surrounding medium as long as the main carbon source in the growth medium was lactate. This observation is in agreement with the data presented in Table 1. Net absorption of ethylene from the surrounding medium decreased on addition of glucose, and eventually the yeast produced significant quantities of ethylene. Addition of glucose also resulted in a faster growth rate. The cell mass more than doubled during a 4-hour incubation with glucose (61 mg to 136 mg), whereas without glucose the increase in cell mass was small (61 mg to 85 mg).

A fairly high concentration of glucose (10.8%) was used in this set of experiments. Its significance in mitochondrial repression and ethylene production will be discussed in a later section.

The shape of the curve in Figure 1 suggests that the rate of ethylene production increased with time after the glucose had been added. It is probable that addition of glucose induced de novo synthesis of enzymes responsible for the synthesis of ethylene. This aspect was studied by addition of cycloheximide along with glucose to the yeast suspension. The results are presented in Figure 2 and Table 2.

Cycloheximide inhibited the glucose-induced ethylene production by the yeast. This suggested that glucose induced the synthesis of an

Fig. 1. Effect of glucose on ethylene production by *Saccharomyces cerevisiae* (X-2180-1B). The yeast was grown in lactate medium to the early log-phase. 20-ml quantities of the culture were used for collection of ethylene. Glucose (10.8%) was added at the time indicated. 20 ml culture contained 61 mg yeast (dry wt)

● No glucose added

○ glucose added

↓ point of addition

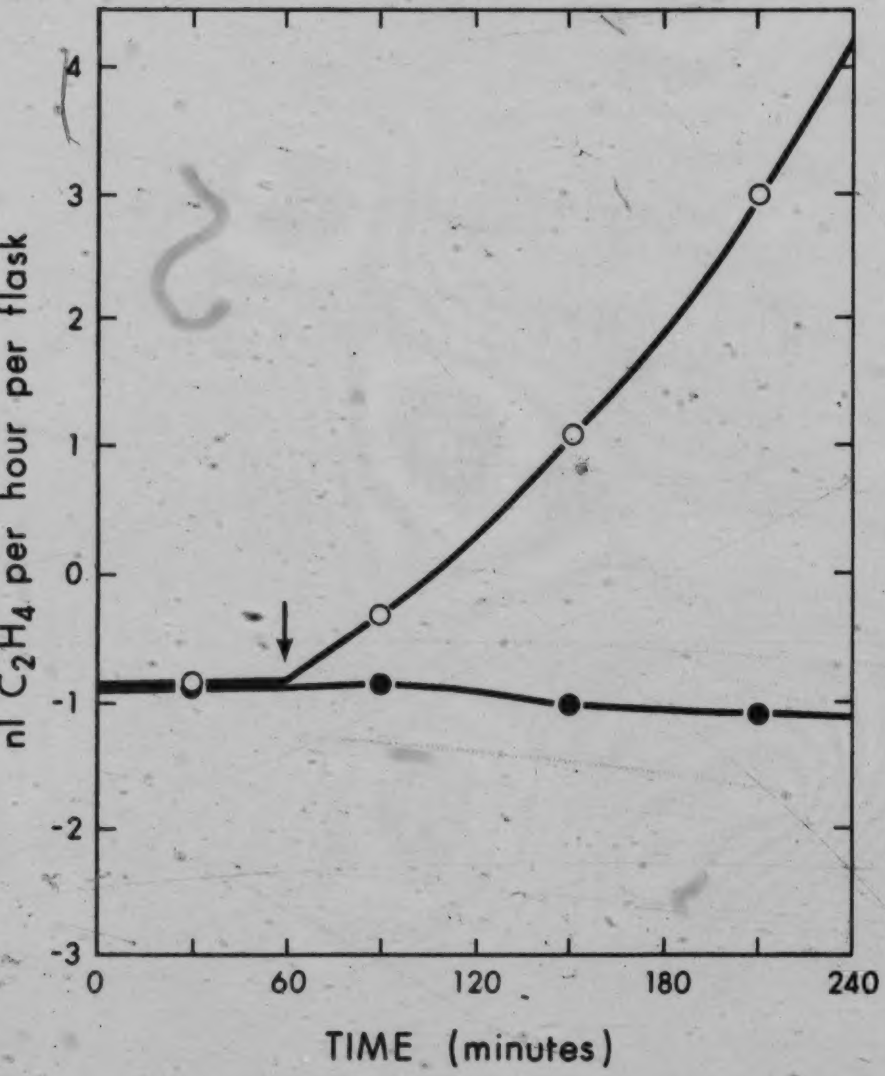

nl C_2H_4 per hour per flask
4
3
2
1
0
-1
-2
-3
0
60
120
180
240
TIME (minutes)

Fig. 2. Effect of cycloheximide on glucose-induced ethylene production by Saccharomyces cerevisiae (X-2180-1B). The yeast was grown to early log-phase in lactate medium. Twenty ml quantities of the culture were used for ethylene collection. Cycloheximide (120 μg/ml) and glucose (10.8%) were added 15 minutes prior to the start of ethylene collection.

○ + glucose

● + glucose + cycloheximide

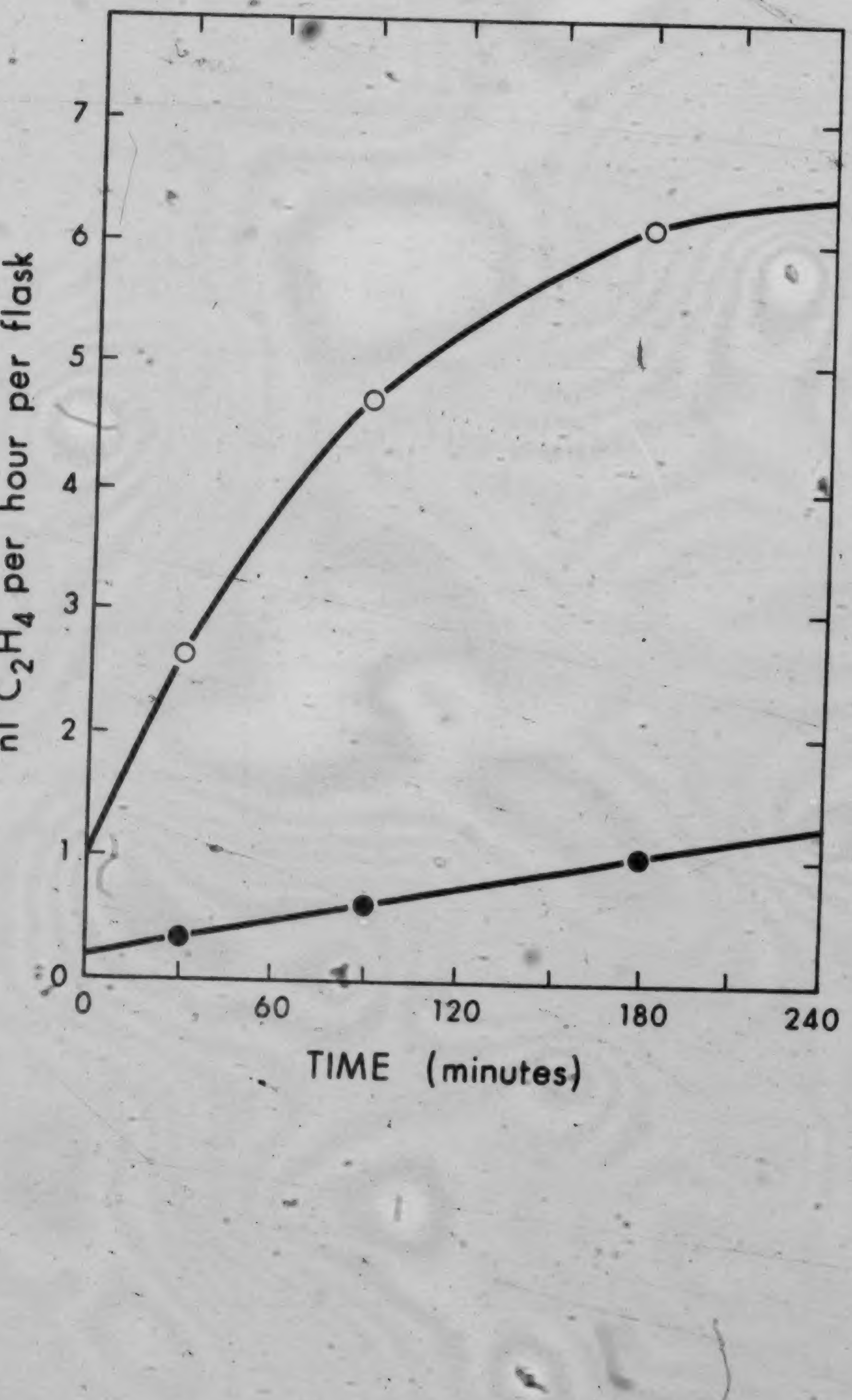

nl C_2H_4 per hour per flask
0
1
2
3
4
5
6
7
0
60
120
180
240
TIME (minutes)

TABLE 2: Effect of Cycloheximide on Glucose-Induced Ethylene Production by *Saccharomyces cerevisiae* (X-2180-1B)

Treatment	Cell Mass (dry wt) at		nℓ Ethylene/4 hr
	0 hr (mg)	4 hr (mg)	
+ Glucose	29	87	19.5
+ Glucose + Cycloheximide	29	34	2.9

The yeast was grown in lactate medium at 27° for 18 hours. Twenty ml portions were distributed in collection flasks, and glucose (10.8%) and cycloheximide (120 µg/ml) were added 15 minutes prior to the beginning of collection period. The results are average values of duplicate determinations.

enzyme or enzymes required for ethylene production. It is interesting to recall in this respect, that cycloheximide inhibits indoleacetic acid-induced ethylene production in pea root tips (36) and in sub-hook segments of etiolated pea epicotyl (83).

Even in the presence of cycloheximide the yeast produces small quantities of ethylene. The rate of production of ethylene in the presence of the inhibitor seems to increase with time as indicated in Figure 2. The effect of cycloheximide on this yeast diminishes with time. Saccharomyces cerevisiae is known to develop resistance to cycloheximide rapidly (121).

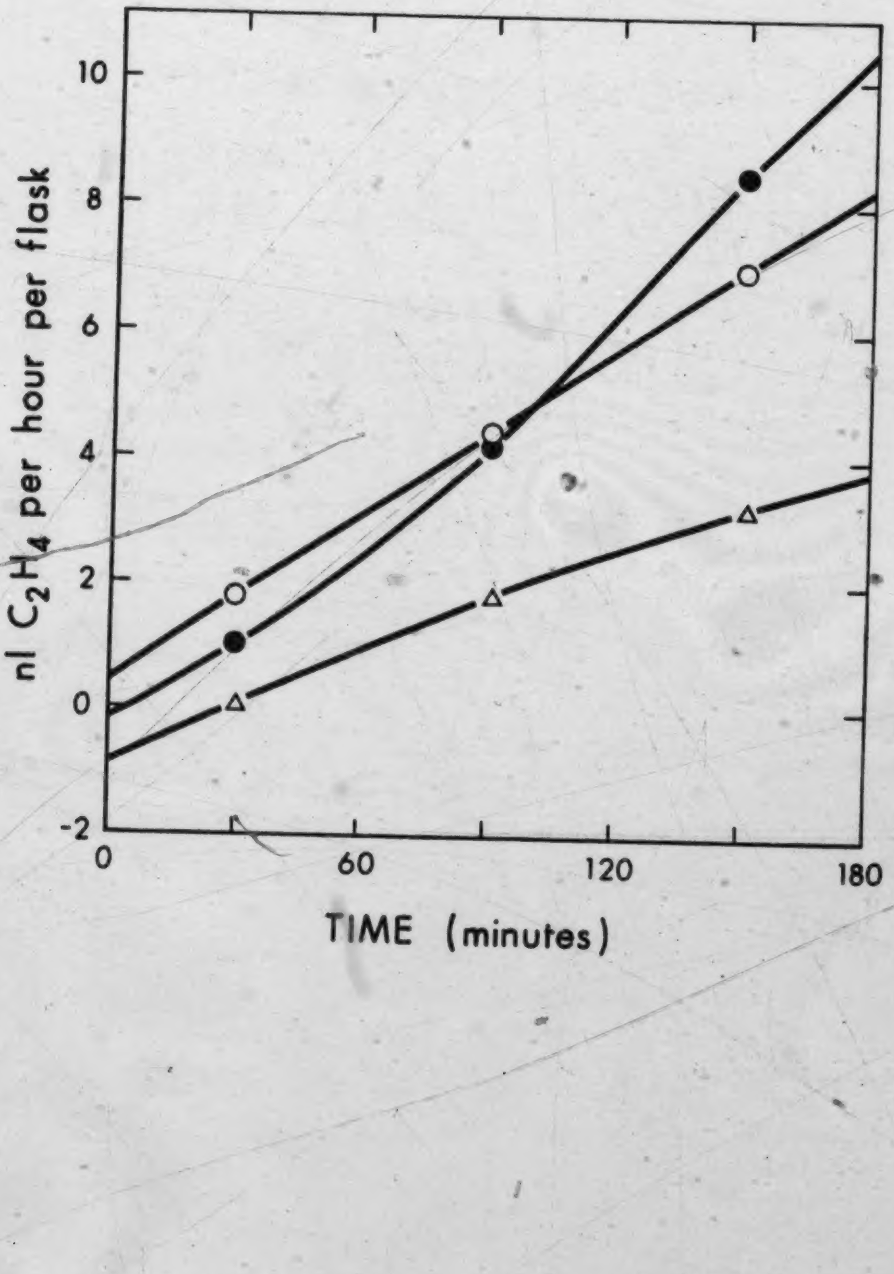
3. Effect of Glucose Concentration on Induction of Ethylene Production

The effect of three concentrations of glucose on induction of ethylene production by the lactate grown yeast (S. cerevisiae X-2180-1B) is shown in Figure 3.

The ethylene production by the yeast during the first hour was inversely proportional to the glucose concentration. At 0.5% (27.75 mM) glucose concentration the ethylene production during the first hour was nearly double that obtained at 1% glucose (54.4 mM). The amount of ethylene produced by the yeast in presence of 10% glucose (544 mM) during the first hour was equal to that evolved by the blank (equal volume of fresh lactate medium containing 10% glucose). On subsequent incubation the rate of production of ethylene continued to increase at all three glucose concentrations, and the maximum production during the third hour of incubation was obtained in the sample that initially contained 1% glucose.

Fig. 3. Effect of glucose concentration on ethylene production by Saccharomyces cerevisiae (X-2180-1B). The yeast was grown in lactate medium to the early log-phase. 20-ml quantities of the culture were used for ethylene collection. Glucose was added 15 minutes prior to the start of ethylene collection. 20 ml culture initially contained 30 mg yeast (dry wt)

○ 0.5% glucose
● 1.0% glucose
△ 10.0% glucose



nl C_2H_4 per hour per flask
10
8
6
4
2
0
-2
0
60
120
180
TIME (minutes)

It appears from these results that the glucose concentration at which the yeast produces the maximum amount of ethylene is 0.5% or less. High concentrations of glucose are known to cause repression in yeast (89). Repression is manifested by the formation of aberrant mitochondria lacking several of the key enzymes required for the transport of electrons from substrates to oxygen. Removal of glucose from the medium results in de-repression. A 10% glucose concentration is known to repress S. cerevisiae completely (89). Even at this concentration of glucose ethylene was being produced by the yeast used in the present study. This suggested that mitochondrial electron transport and oxidative phosphorylation per se were not essential requirements for ethylene production. However, ethylene production by S. cerevisiae (X-2180-1B) seemed to be maximum when glucose-induced repression was minimum. For example, at a concentration of 0.5% glucose the repression was less than that a 1% glucose and the ethylene production was more in the former case than in the latter. With prolonged incubation the concentration of the sugar in the sample that initially contained 1% glucose, reached an optimum level at which ethylene production was maximum. It may be concluded from this experiment that biosynthesis of ethylene by Saccharomyces cerevisiae (X-2180-1B) is stimulated by small quantities of glucose, and the optimal rate of production of the volatile is favoured by a non-repressing concentration of the sugar.

4. Effect of Oxygen on Ethylene Production

Whether respiration and mitochondrial ATP formation are obligatory to ethylene biogenesis is not known. This aspect was studied using respiration deficient mutants isolated from the wild yeast S. cerevisiae (X-2180-1B). The results are presented in Table 3.

TABLE 3: Ethylene Production by Respiration Deficient Mutants Isolated from Wild Type Saccharomyces cerevisiae (X-2180-1B)

Mutant	Cell Mass (dry wt) at		Ethylene Production	
	0 min (mg)	120 min (mg)	0-60 min (nℓ)	60-120 min (nℓ)
A	57	84	4.3	4.4
B	124	151	14.3	15.1

The mutants were grown in glucose medium for 16 hours. Twenty ml portions were distributed in collection flasks, and the ethylene collected as described in Materials and Methods. The results are average values of duplicate determinations.

It can be seen that respiration deficient mutants produced relatively large quantities of ethylene. Since these mutants lacked funcational mitochondria, they did not respire. They obtained their metabolic energy by fermenting glucose, and therefore, energy required for ethylene synthesis also might have come from this catabolic process.

While this experiment tentatively proved that ATP formed by the mitochondrial oxidative phosphorylation was not essential for ethylene biogenesis, it still did not provide evidence against participation of molecular oxygen in the biosynthetic pathway of ethylene. Since the mutants were grown and the rate of ethylene production determined under aerobic conditions, it was quite possible that molecular oxygen did take part in the biosynthesis of ethylene. This was studied by growing the wild yeast (S. cerevisiae X-2180-1B) under anaerobic conditions, and determining the rate of ethylene production in the absence and presence of oxygen. The results are presented in Figure 4.

Anaerobically grown yeast and filtrate obtained from it produced significant quantities of ethylene in the obsence of oxygen. The rates of ethylene production by filtrate and medium containing yeast increased rapidly on admission of air. This suggested that under anoxic conditions the rate of conversion of precursor to ethylene was slow, and on admitting oxygen to the medium this conversion was stimulated.

The rate at which ethylene was being produced leveled off shortly after exposing the yeast to aerobic conditions, whereas evolution of ethylene from filtrate under the same conditions delcined after an initial rise. This declining rate of evolution of ethylene from the filtrate might result from the exhaustion of precursor or cofactor that apparently was produced by the yeast.

Fig. 4. Ethylene production by anaerobically grown Saccharomyces cerevisiae (X-2180-1B) and the effect of oxygen on ethylene production. The yeast was grown in glucose medium. Anoxic conditions were maintained by bubbling sterile nitrogen through the medium. Air was admitted at the time indicated.

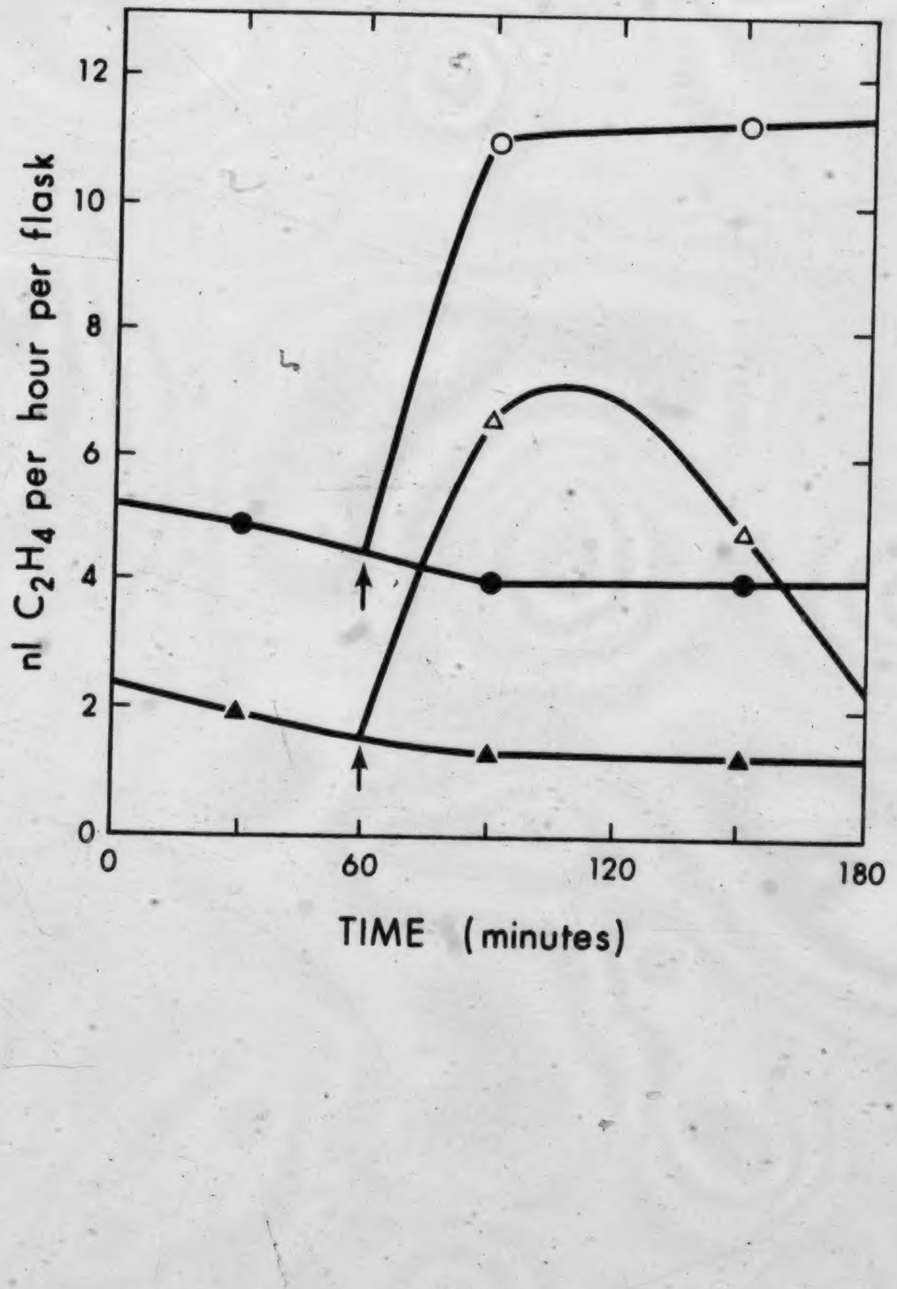
● Production of ethylene by yeast, anaerobic.

○ Production of ethylene by yeast, aerobic.

△ Production of ethylene by filtrate, aerobic.

▲ Production of ethylene by filtrate, anaerobic.

↑ Point of admitting air


nl C_2H_4 per hour per flask
12
10
8
6
4
2
0
0
60
120
180
TIME (minutes)

5. Effect of pH on Ethylene Production

The following experiment was conducted to study whether the pH of the external medium influences the rate of production of ethylene by Saccharomyces cerevisiae (X-2180-1B). Uninoculated medium had a pH of 4.8 and it dropped to 3.6 after growing the yeast in it for 16 hours. At 16 hours half of the culture was filtered and aliquots of the filtrate were adjusted to pH 4.0, 5.0 and 6.0 with 1 M KOH. Aliquots of the other half of the culture were also similarly adjusted. Rates of ethylene production by these samples were determined; the results presented in Table 4 are average values of duplicate determinations.

Maximum amounts of ethylene were produced by the filtrate and by medium containing the yeast when the pH had been adjusted to 4.0. Samples adjusted to pH 5.0 evolved considerably less ethylene and the difference between pH 5.0 and 6.0 was not very marked. The pH of the filtrate did not change appreciably during the ethylene collection period. In the presence of yeast, however, there was a drastic decrease of the pH of the media which had been adjusted to pH 4.0 and 5.0. The drop in pH of the yeast-containing medium that had an initial pH of 6.0 was less marked. In this case, the change in pH of the culture was slightly more than that of the filtrate after 2 hours, although for the culture it did take more KOH in the initial adjustment of the pH.

It appears from these results that maximum ethylene production from filtrate, and medium containing yeast, is favored by a pH around 4. However, ethylene production by the yeast seems to be unaffected by the adjustment of the pH of the medium. This is shown in Figure 5.

It might be argued, however, that the yeast grew and lowered the pH of the medium from an initial value of 5.0 to 3.8 (Table 4) and

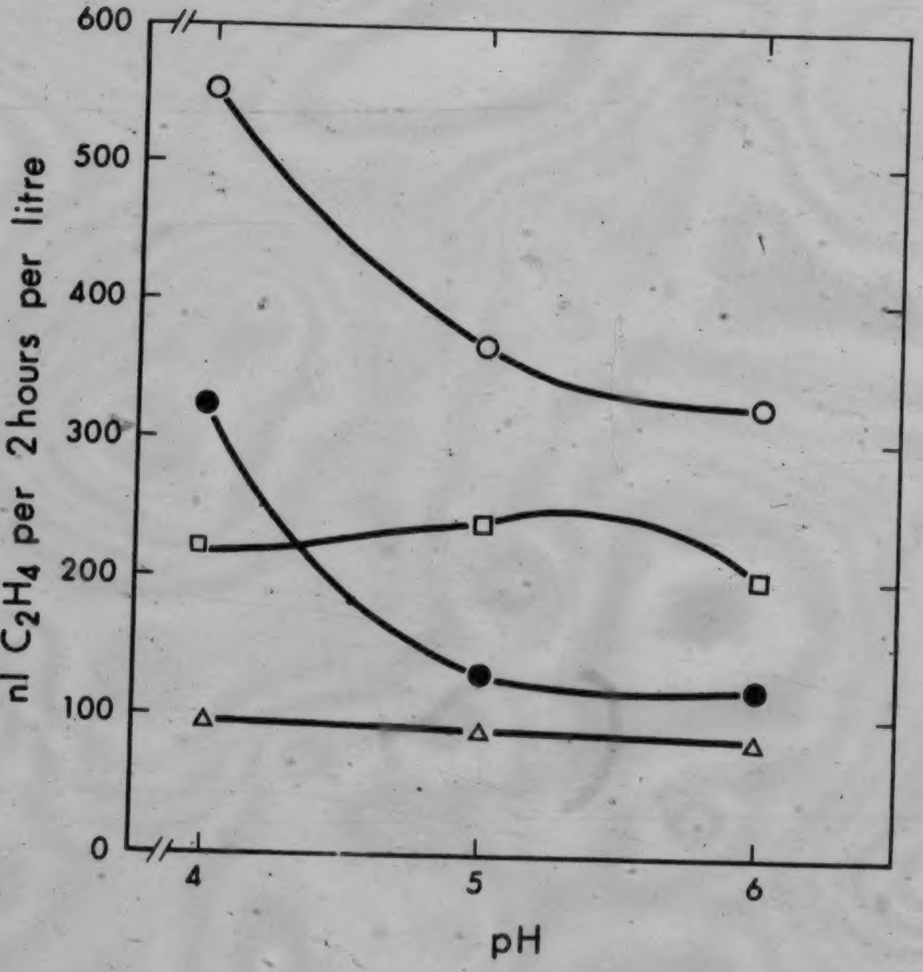
TABLE 4: Effect of pH on Ethylene Production by *Saccharomyces cerevisiae* (X-2180-1B)

Sample	pH			M. Equiv. KOH used/Litre	Total Ethylene /litre/2 hr (nℓ)
	Initial (0 hr)	Adjusted to	Final (2 hr)		
Filtrate	3.6	4.0	4.0	10.0	327
	3.6	5.0	5.1	30.0	131
	3.6	6.0	6.2	92.5	120
Yeast Culture	3.6	4.0	3.3	10.0	550
	3.6	5.0	3.8	30.0	368
	3.6	6.0	5.7	105.0	322

The yeast was grown at 27° in glucose medium for 16 hours. Half of the culture was filtered through Millipore membrane filter (0.8 μ). Aliquots of the filtrate were adjusted to pH 4.0, 5.0 and 6.0 with 1.0 M KOH. Similarly aliquots of the yeast culture also were adjusted.

Fig. 5. Effect of pH on ethylene production. Saccharomyces cerevisiae (X-2180-1B) was grown in glucose medium for 16 hours. The pH of the culture and the filtrate obtained from it were adjusted with KOH. The pH of the fresh medium was adjusted with KOH or H_3PO_4.

- ○ Yeast culture
- ● Filtrate
- □ Difference between yeast culture and filtrate
- △ Fresh medium


600
500
400
300
200
100
0
nl C_2H_4 per 2 hours per litre
4
5
6
pH

this decreased pH was responsible for the increased production of ethylene. This does not seem to be the case. When the pH of the medium containing the yeast was adjusted to 6.0, the ethylene production was still comparable to that produced by the sample adjusted to pH 5.0, even though the pH dropped only to 5.7 in the former.

Uninoculated medium produced small amounts of ethylene. This production was not dependent on the pH of the medium (Figure 5). Therefore, the uninoculated medium did not seem to contain 'the precursor' whose conversion to ethylene is favored by a lower pH. It may be concluded that the 'precursor' is produced by the yeast and passed into the medium.

6. Effect of L-methionine on Ethylene Production

The effect of L-methionine of ethylene production by *Saccharomyces cerevisiae* (X-2180-1B) was studied and the results are presented in Figure 6.

The rate of production of ethylene by the yeast in lactate medium was less than that produced by the blank (uninoculated medium). On addition of L-methionine (1 mM), the ethylene production by the yeast-containing medium increased, and eventually reached a value greater than that produced by the blank.

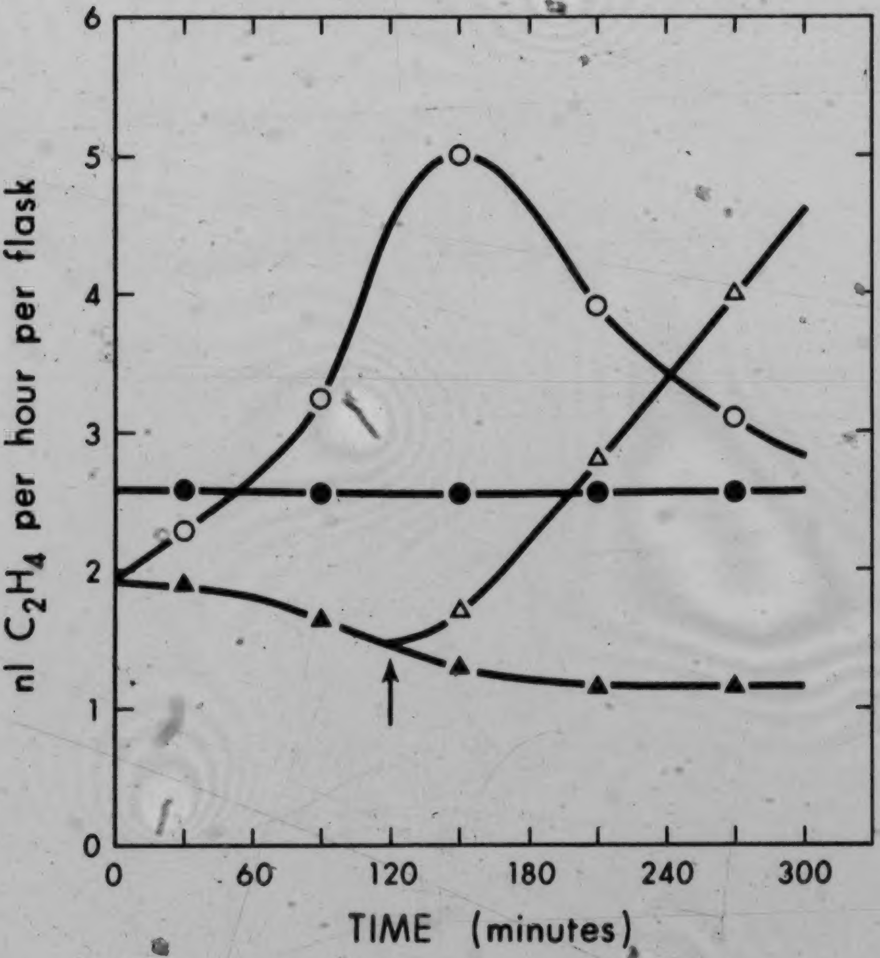
Ethylene production by the lactate grown yeast was also increased by the addition of glucose (2% at zero time). This is in agreement with the results presented in Figures 2 and 3. The rate of production of ethylene was maximal by the third hour; thereafter the rate seemed to decrease. This may result from the exhaustion of glucose from the medium.

7. Synergistic Effects of D-glucose and L-methionine on Ethylene Production

Previous experiments have shown (Figure 6) that D-glucose and

Fig. 6. Production of ethylene by Saccharomyces cerevisiae (X-2180-1B) in the presence of glucose or L-methionine. The yeast was grown in lactate medium to the mid-log phase. Forty ml quantities of the culture were used for ethylene collection. Glucose (2%) was added at zero time. L-Methionine (1mM) was added 2 hr after the start of ethylene collection.

▲ Yeast growing in lactate medium
△ L-methionine added to the yeast growing in lactate medium
○ Glucose added to the yeast growing in lactate medium
● Fresh uninoculated medium
↑ Point of addition of methionine


nl C_2H_4 per hour per flask
TIME (minutes)
0
60
120
180
240
300
0
1
2
3
4
5
6

L-methionine stimulate ethylene production by S. cerevisiae (X-2180-1B) independently. In the following experiments the synergistic effects of these two compounds on ethylene production by the yeast were studied. The results are presented in Figures 7 and 8 and in Table 5.

In the first case, L-methionine was added to the lactate grown yeast culture at the beginning of the ethylene collection period (see Figure 7). Immediately after the addition of L-methionine, the rate of production of ethylene started to increase and within a few hours it leveled off. When glucose was added to the yeast culture which had previously been treated with L-methionine, the rate of ethylene production increased rapidly. Two hours after the addition of glucose the ethylene production had increased over four-fold compared to the sample that contained L-methionine but not glucose. Stimulation by D-glucose or L-methionine alone of ethylene production was small, but when they were present together in the growth medium, there was a tremendous increase in the amount of ethylene synthesized by the yeast (compare Figures 6 and 7).

It was found in earlier experiments that when the growth medium contained low concentrations of glucose, the medium became depleted of glucose by growing yeast within a few hours. Therefore, in this experiment (Figure 7) the addition of glucose to the yeast culture was delayed for 2 hours to allow methionine-induced stimulation of ethylene production to reach a peak value.

In the following experiment the order of addition of methionine and glucose was reversed. Glucose was added at the beginning of the ethylene collection period, and L-methionine after 2 hours. The results are presented in Figure 8. Addition of glucose to the lactate-grown yeast

Fig. 7. Effect of glucose and L-methionine on ethylene production by Saccharomyces cerevisiae (X-2180-1B). The yeast was grown in lactate medium to the mid-log phase. Forty ml quantities of the culture were used for ethylene collection. L-Methionine (1mM) was added at zero time and glucose (2%) was added 2 hours after the addition of L-methionine. The values were subtracted from the corresponding blank (fresh medium) values.

△ Ethylene production by the yeast in lactate medium

○ Ethylene production by the yeast in lactate medium that contained 1mM L-methionine

● Ethylene production by the yeast in lactate medium that contained 2% glucose and 1mM L-methionine

↓ Point of addition of glucose

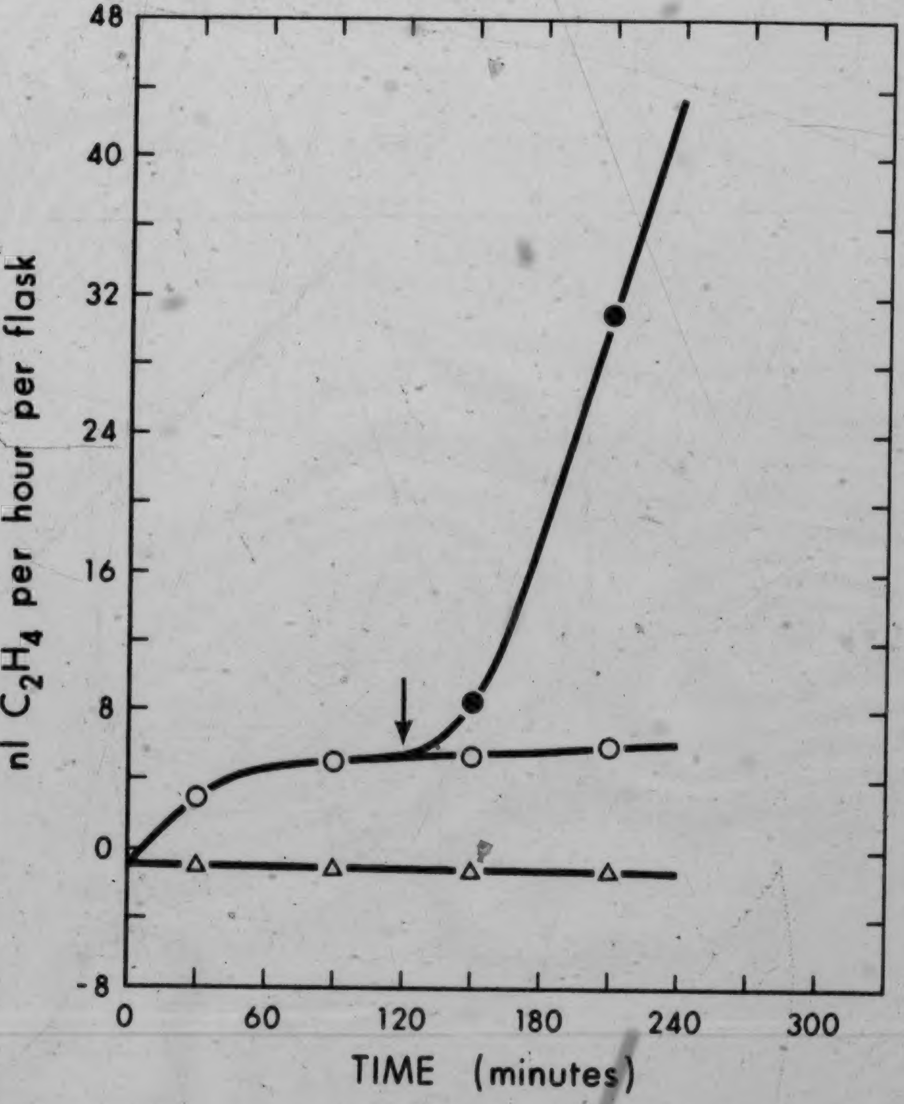
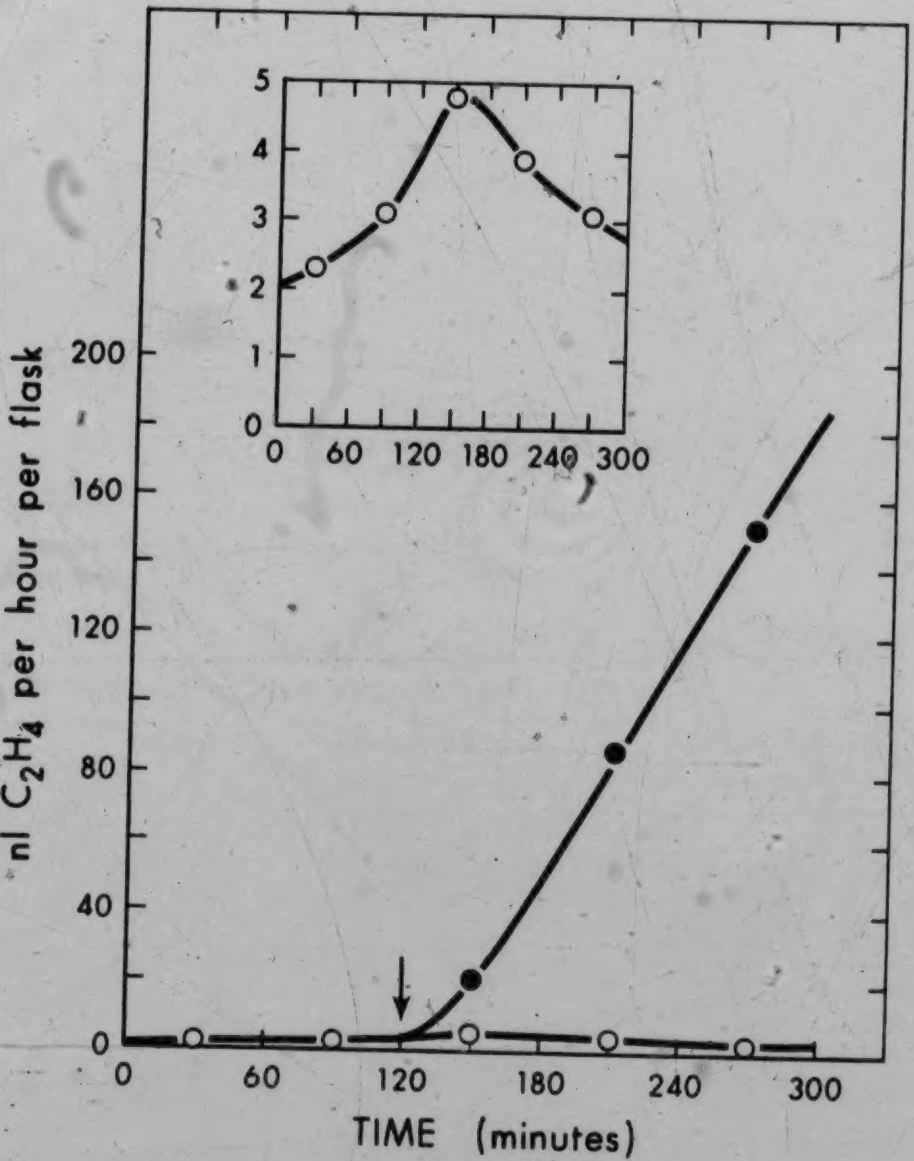

48
40
32
24
16
8
0
-8
nl C_2H_4 per hour per flask
0
60
120
180
240
300
TIME (minutes)

Fig. 8. Effect of glucose and L-methionine on ethylene production by Saccharomyces cerevisiae (X-2180-1B). The yeast was grown in lactate medium to the mid-log phase. Forty ml quantities of the culture were used for ethylene collection. Glucose (2%) was added at zero time and L-methionine (1mM) was added 2 hours after the addition of glucose. The inset indicates the rate of ethylene production in presence glucose on a magnified scale.

○ Ethylene production in presence of glucose

● Ethylene production in presence of glucose and L-methionine

↓ Point of addition of methionine


nl C_2H_4 per hour per flask
200
160
120
80
40
0
0
60
120
180
240
300
TIME (minutes)
5
4
3
2
1
0
0
60
120
180
240
300

increased ethylene production by 3.8-fold over control within 3 hours. On further incubation, however, the rate of production of ethylene declined, and by the end of 5 hours the increased ethylene production was only 2.7-fold over the control. (See the inset in Figure 8.) Addition of 1 mM L-methionine at the end of 2 hours to the glucose containing yeast culture increased the ethylene production over 130-fold within 3 hours. This showed that glucose and methionine had a synergistic effect on stimulation of ethylene production by S. cerevisiae (X-2180-1B).

The rate of production of ethylene by the yeast in the presence of glucose and methionine is very large compared to any plant tissue. On a per gram basis this yeast produced 4.6 μl of ethylene per hour. Comparable value per hour for apple (a high ethylene producer) is 0.03 μl per gram fresh weight (86).

It has been shown by tracer studies that apples, banana and pea stems produce ethylene from methionine (28). Mapson (108) suggested that the conversion of methionine to ethylene was facilitated by peroxide generated by the action of glucose oxidase on D-glucose. Although it has not been conclusively proven that the stimulatory effect of glucose on the conversion of methionine to ethylene is through generation of peroxide, there is no doubt that for maximal production of ethylene from methionine by yeast, the presence of glucose also is needed. The large stimulation of ethylene production by L-methionine in the presence of glucose suggests that glucose participates in the biosynthesis of ethylene in S. cerevisiae (X-2180-1B).

8. Changes in the pH of the Medium, Cell Mass and Rate of Ethylene Production

When the growth medium did not contain glucose or methionine and the main carbon source in the medium was lactate, the cell mass of the yeast increased from 33 mg to 86 mg during an interval of five hours (Table 5). Ethylene production by this sample was low. Addition of L-methionine (1 mM) at 120 minutes did not alter the growth pattern, but ethylene production was stimulated seven fold. On the other hand, if glucose was added (2% glucose was added to ensure that glucose was present throughout the duration of the experiment) the cell mass increased to 178 mg from an initial 33 mg in 5 hours and ethylene production increased 8-fold over control. L-methionine did not affect the glucose-stimulated growth of the yeast, but increased ethylene production over 130-fold (compared to the control).

Changes in pH of the medium brought about by the addition of glucose, methionine, and glucose plus methionine are worth noting. Yeast grown on lactate increased the pH of the medium from 5 to 6.1, and addition of L-methionine (1 mM) did not seem to affect this increase in pH. Addition of glucose (2%) decreased the pH from 5 to 4.5 and even in the presence of glucose, methionine did not have an effect on pH change.

Two explanations for the increase in the pH of the medium in which the yeast was grown without added glucose are: (a) Hydrogen ion from the medium was taken up by the yeast; (b) Carbon dioxide from the medium was absorbed by the yeast, thus shifting the equilibrium of the reaction shown below to the left.

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$$

When the yeast is grown in lactate medium the first product that is derived from lactate by the yeast is pyruvate. Pyruvate serves as a precursor in the biosynthesis of a great number of cell constituents.

TABLE 5: Changes in pH, Cell Mass, and Rate of Ethylene Production on Addition of Glucose and L-Methionine

Sample	pH at		Cell Mass (dry wt) at		Ethylene Production	
	0 min	300 min	0 min (mg)	300 min (mg)	0-120 min (nℓ)	120-300 min (nℓ)
Fresh medium (blank)	4.7	4.7	-	-	5.2	7.6
Control	5.0	6.1	33	86	1.6	2.3
2% glucose at 0 min	5.0	4.5	33	178	10.8	19.7
1 mM L-methionine at 120 min	5.0	6.2	33	90	1.8	16.1
2% glucose at 0 min + 1 mM L-methionine at 120 min	5.0	4.5	33	182	10.6	263.0

The yeast was grown in lactate medium at 27° for 18 hours. Forty ml quantities of the yeast culture were distributed in collection flasks. Glucose and L-methionine were added at indicated times during the collection period.

Also, oxidation of pyruvate through tricarboxylic acid (TCA) cycle yields energy for cell metabolism. Operation of TCA cycle is important on two counts. (a) It yeilds energy needed for the survival and growth of the organism. (b) It provides intermediates required for the synthesis of several of the amino acids. Draining of TCA cycle intermediates for amino acid synthesis cannot occur for an extended length of time without shutting off the cycle. Splittstoesser (166) suggested that this difficulty could be overcome by dark fixation of CO_2 involving malic enzyme or phosphoenolpyruvate (PEP) carboxylase. Products of dark fixation of CO_2 (malic acid and oxaloacetic acid) are intermediates of TCA cycle. Therefore, as long as dark fixation of CO_2 continues, the biosynthetic role of the TCA cycle will not be inhibited. Recently Bown and Aung (24) and Bown et al. (25) have shown that dark fixation of CO_2 takes place in plants even at ambient levels of CO_2 and it is vital to plant growth.

It is reasonable to expect that when most of the amino acids are to be synthesized from pyruvate, as in the present study, dark fixation of CO_2 is an essential feature of cell matabolism. Hence it may be reasoned that the increase in pH observed when the yeast is grown in lactate medium is a result of the removal of CO_2 from the medium.

When glucose is present in the medium, carbon dioxide is produced by the yeast in large quantities through fermentation. Carbon dioxide, in turn, may lower the pH of the medium. Also, in yeast K^+ is co-transported with glucose and in exchange H^+ is excreted into the medium (174). This also may contribute to the lowering of the pH of the medium. Thus even if dark fixation of CO_2 takes place in the glucose-grown yeast, it may not manifest itself by increasing the pH of the medium.

9. Effect of L-methionine on Production of Ethylene by the Filtrate of Glucose-Grown Yeast

Saccharomyces cerevisiae (X-2180-1B) was grown in a glucose-containing medium for 18 hours and the cells removed by filtering through a Millipore membrane filter (0.8 pore size). Rates of production of ethylene by the filtrate in the presence of L-methionine (1 mM) and in its absence were determined. The results presented in Figure 9 are average values of [illegible]licate determinations.

The rate of production of ethylene from the filtrate that did not contain L-methionine decreased slightly with time. On the other hand, there was a small increase in the amount of ethylene produced by the filtrate containing L-methionine. It is not certain whether the increased ethylene production is a result of enzymatic conversion of L-methionine to ethylene. (Addition of L-methionine to uninoculated medium did not increase ethylene production.) If the conversion is enzymatic in nature, then activity of the enzyme(s) responsible for this conversion is extremely low. On the other hand, the conversion may be non-enzymatic; something similar to that described in certain model systems (87). In the presence of the yeast, production of ethylene from methionine is very rapid (Figure 8). Whatever the mechanims of conversion of methionine to ethylene in the filtrate may be, it cannot account for the fast production of ethylene from methionine when the medium contains the yeast.

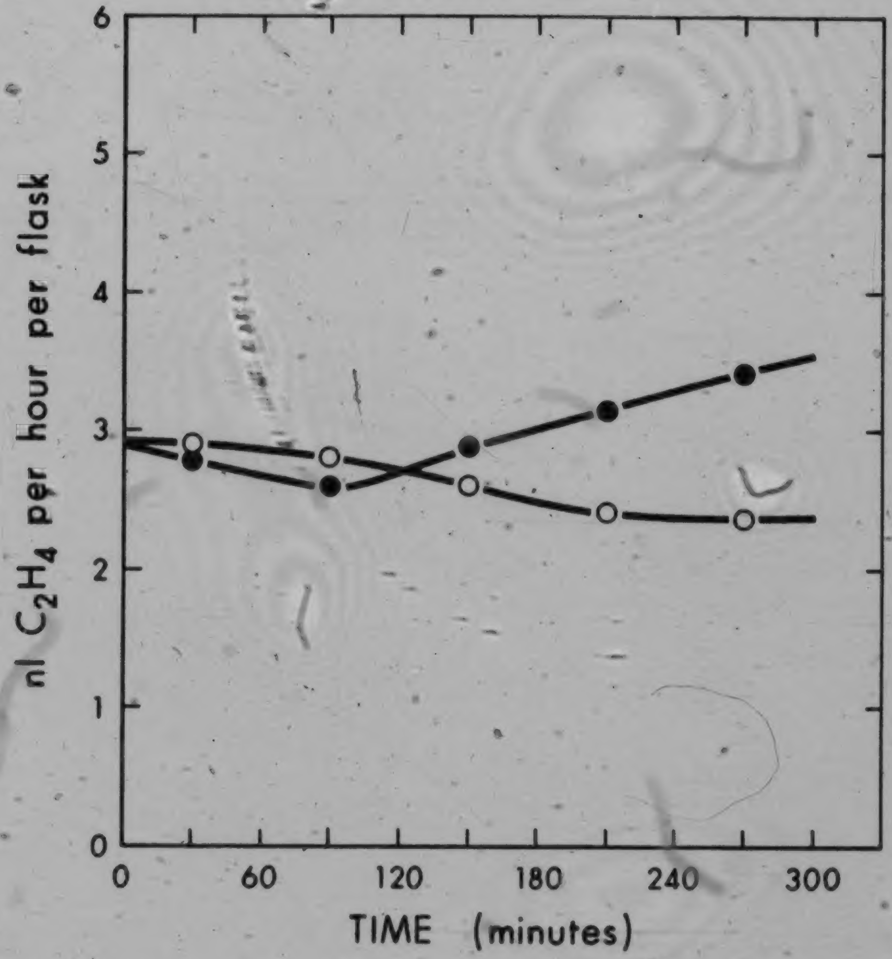
10. Effect of L-methionine Concentration of Ethylene Production of Saccharomyces cerevisiae (X-2180-1B)

In all previous experiments (Figures 6, 7, 8 and 9, and Table 5) L-methionine was used at a concentration of 1 mM. In the following experiment

Fig. 9. Effect of L-methionine on ethylene production by cell-free growth medium. Saccharomyces cerevisiae was grown in glucose medium to the mid-log phase. The culture was filtered and 40 ml quantities of the filtrate were used for ethylene collection. L-Methionine (1mM) was added to the filtrate at the beginning of ethylene collection period.

○ Filtrate

● Filtrate + L-methionine


nl C_2H_4 per hour per flask
0
1
2
3
4
5
6
0
60
120
180
240
300
TIME (minutes)

the effect of L-methionine concentration on ethylene production by the yeast was studied (Figure 10). Since glucose was found to be essential for a maximal rate of ethylene production, 2% glucose (w/v) was added to each sample at the beginning of the ethylene collection period. Various concentrations of L-methionine were added two hours after the addition of glucose.

Ethylene production was stimulated at all three concentration levels of L-methionine, in comparison to the control without methionine. However, the rate of production of ethylene at the lowest concentration of L-methionine (0.2 mM) leveled off within 2 hours after the addition of the amino acid. At higher concentrations of L-methionine (1 mM and 5 mM) ethylene production continued to increase.

The endogenous concentration of free L-methionine in plant tissues that produce large amounts of ethylene is very low (15). Therefore, to maintain a high rate of ethylene production, methionine must be synthesized at a fast rate (14), or methionine must be exogenously applied (86). Intracellular concentration of free methionine in S. cerevisiae grown in the absence of any added methionine is reported to be less than 2 μmoles per gram dry weight, and the value could go as high as 120 μmoles per gram dry weight if the suspending medium contained 20 mM l-methionine (51). Lor and Cossins (90) have suggested that L-methionine controls the methyl group biogenesis within the folate pool of Saccharomyces and exogenously applied methionine (2.5 mM) inhibits its won synthesis.

In the present study, however, the growth rate of the yeast was not affected by methionine at any of the concentrations used. Even at 5 mM concentration the 'adenine trapping effect' (50) of methionine did

Fig. 10. Effect of L-methionine concentration on ethylene production by *Saccharomyces cerevisiae*(X-2180-1B). Forty ml quantities of the lactate grown yeast were used for ethylene collection. Glucose was added at zero time and various concentrations of L-methionine were added 2 hours after the addition of glucose.

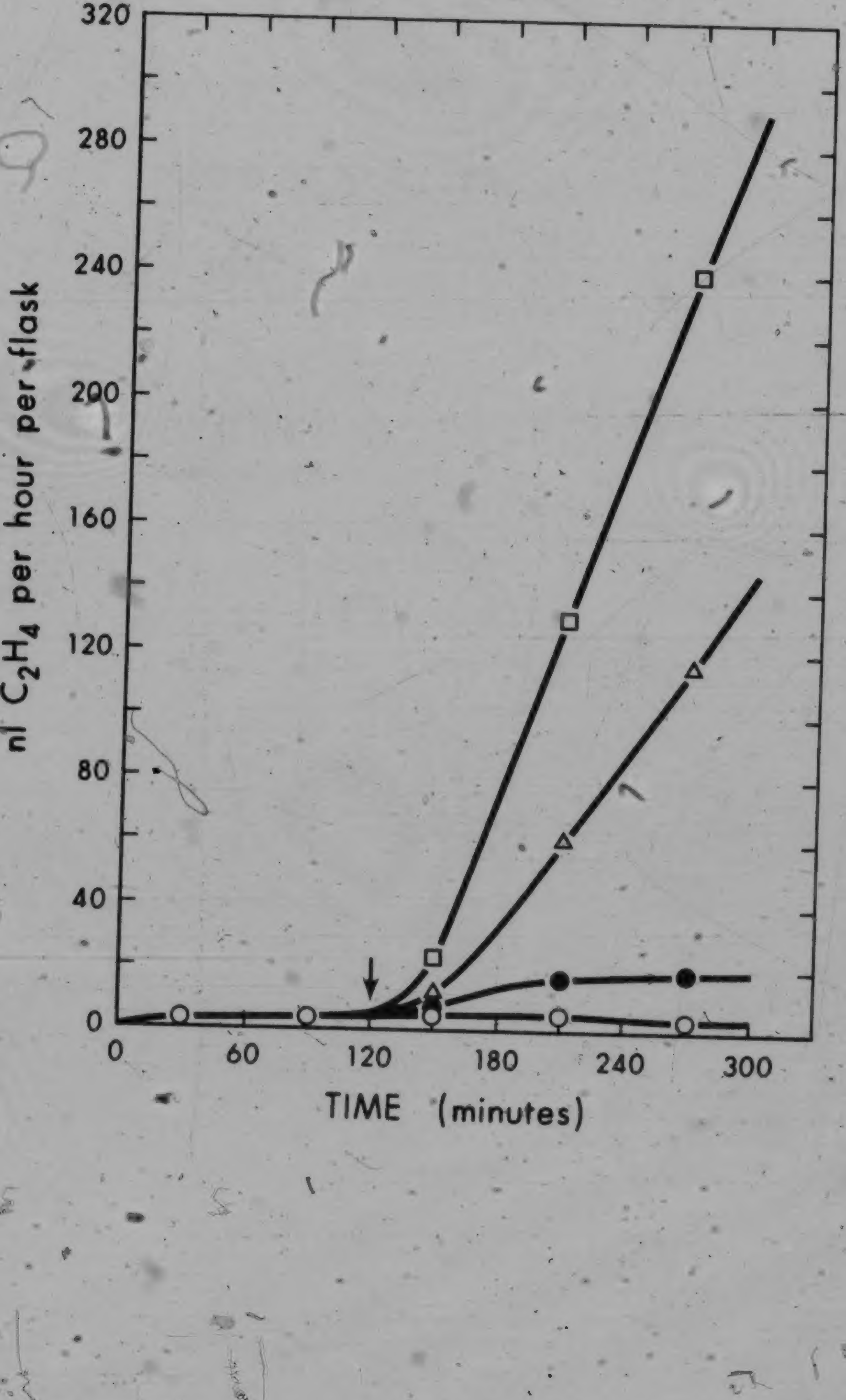
○ No methionine added

● 0.2 mM L-methionine

△ 1.0 mM L-methionine

□ 5.0 mM L-methionine

↓ point of addition of methionine


nl C_2H_4 per hour per flask
320
280
240
200
160
120
80
40
0
0
60
120
180
240
300
TIME (minutes)

not manifest itself in decreased growth rate.

Since exogenously applied methionine while inhibiting its own synthesis (90), stimulates ethylene production as shown in the present study, it may be inferred that the methionine-stimulated ethylene production does not involve the intermediates of methionine biosynthetic pathway.

11. Effects of D-methionine and L-ethionine on Ethylene Production by Saccharomyces cerevisiae (X-2180-1B)

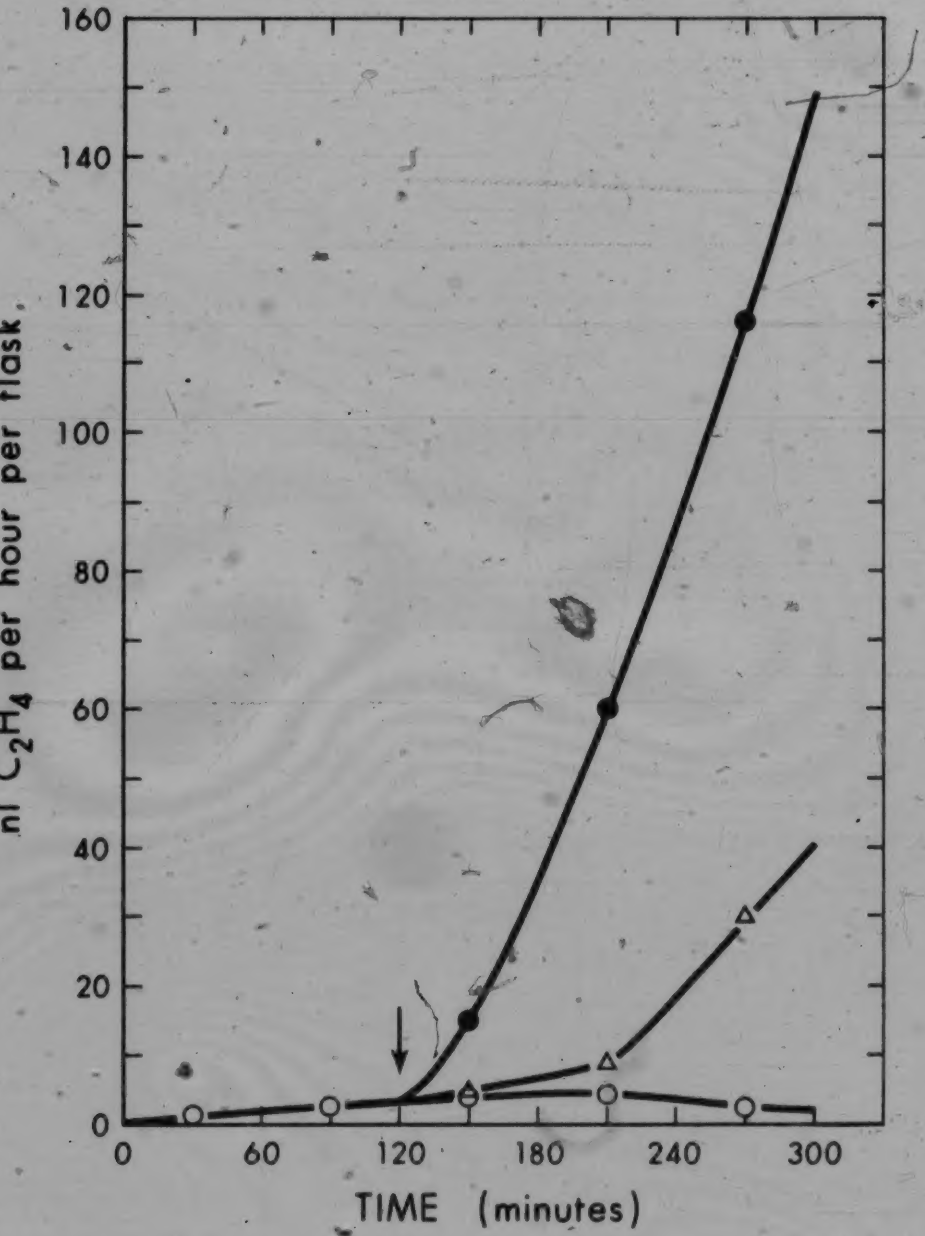
Mapson (108) suggested that the conversion of methionine to ethylene in model systems is dependent on the presence of the structure $R-S-CH_2-CH_2-$, where R group should be either CH_3 or C_2H_5. D-methionine and L-ethionine should yield ethylene with equal efficiency to L-methionine in an ethylene producing biological system provided any enzymes involved were not capable of distinguishing between them.

In most organisms, D-methionine is a non-metabolizable substrate that cannot replace L-methionine. L-ethionine is a competitive inhibitor of transmethylation reactions involving L-methionine (41). Occurrence of D-methionine in plants has been demonstrated but ethionine in an analogue of methionine that does not occur naturally.

The effect of D-methionine on ethylene production by the yeast is shown in Figure 11. While the ethylene production increased immediately after the addition of L-methionine (1 mM), in the presence of D-methionine (1 mM) the ethylene production started to increase only after a lag of two hours. This suggested that either induction of D-methionine permease was slow, or D-methionine had to be converted to L-methionine before it could yield any ethylene. Such a conversion of D-methionine to its L-isomer has been reported in rat and man (121). It is not certain whether such a conversion takes place in yeast.

Fig. 11. Effect of L-methionine and D-methionine on ethylene production by Saccharomyces cerevisiae (X-2180-1B). Forty ml quantities of lactate grown yeast were used for ethylene collection. Glucose (2%) was added at zero time and L-methionine (1mM) and D-methionine (1mM) were added 2 hours after the addition of glucose.

- ○ Control (no D- or L-methionine)
- △ 1 mM D-methionine
- ● 1 mM L-methionine
- ↓ Point of addition of methionine


nl C_2H_4 per hour per flask
160
140
120
100
80
60
40
20
0
0
60
120
180
240
300
TIME (minutes)

The model (108) that suggests that ethylene is derived from the C_3-C_4 moiety of L-methionine does not give an explanation as to the fate of methyl group attached to the sulphur atom. Burg (26) suggested that the methyl group enters the C_1 pool. This suggestion implicates the involvement of a transmethylation reaction in the production of ethylene from L-methionine. If such is the case, the conversion of L-methionine to ethylene should be inhibited by L-ethionine, which is known to be a competitive inhibitor of transmethylation reactions involving L-methionine (41).

The results presented in Figure 12 are not in agreement with such a proposal. When both L-methionine and L-ethionine were present in equal concentrations (1 mM each) the ethylene production was more than that obtained in the presence of either compound alone. Blanks containing L-ethionine did not produce significant amounts of ethylene. Increasing the ratio of L-ethionine to L-methionine to 3:1 further increased the ethylene production. These experiments showed that ethylene production was enhanced when L-methionine and L-ethionine were added singly or together. If methionine was converted to ethylene, the pathway of conversion probably did not require transmethylation of L-methionine as a prior step.

As long as methionine was also present in the medium, L-ethionine did not affect the growth of the yeast. But in the absence of L-methionine a concentration of 1 mM l-ethionine inhibited the growth of the yeast by 34% in 3 hours. In spite of the inhibition of the growth, ethylene production in yeast continued to be stimulated by L-ethionine.

It is not certain whether L-ethionine is directly converted to ethylene, or it is first transformed to L-methionine. In this respect,

Fig. 12. Effect of L-methionine and L-ethionine on ethylene production by *Saccharomyces cerevisiae* (X-2180-1B). Forty ml quantities of lactate grown yeast were used for ethylene collection. Glucose (2%) was added at zero time. L-Methionine and L-ethionine were added 2 hours after the addition of glucose.

○ Control

△ 1 mM L-ethionine

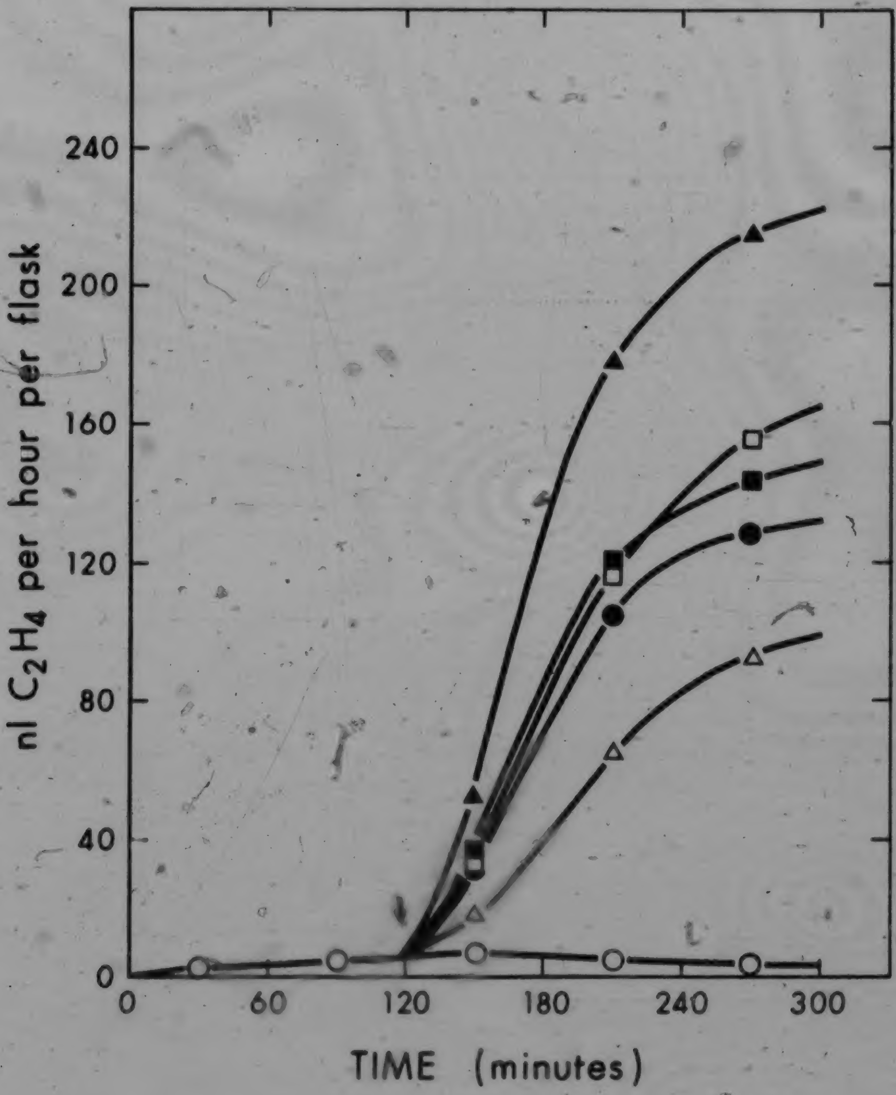
● 1 mM L-methionine

■ 1 mM L-methionine + 0.5 mM L-ethionine

□ 1 mM L-methionine + 1 mM L-ethionine

▲ 1mM L-methionine + 3 mM L-ethionine

↓ point of addition


nl C_2H_4 per hour per flask
0
40
80
120
160
200
240
0
60
120
180
240
300
TIME (minutes)

it can be recalled that, according to Maw (114), as much as 30% of the sulphur of ethionine can be found in methionine residues after incorporation of ethionine into yeast protein.

12. Inhibition of Ethylene Production by Pyruvate

Durham et al. (49) suggested that L-methionine, before being converted to ethylene was transaminated and the resulting α-keto analogue of methionine was converted to ethylene by a free radical mechanism. They isolated and purified from germinating peanut seedlings, an amino transferase that was specific for L-methionine. The most effective amino acceptor for this transferase was found to be pyruvate.

If the contention of these authors is correct, pyruvate must be an active participant in the formation of ethylene from L-methionine. This aspect was studied by adding potassium pyruvate (1 mM) along with L-methionine (1 mM) to the yeast S. cerevisiae (X-2180-1B), and following the rate of ethylene production. The results are presented in Figure 13.

In the absence of added pyruvate, the stimulation of ethylene production by L-methionine followed the pattern described earlier (Figure 7). In the presence of added pyruvate, however, stimulation of ethylene production by L-methionine was not evident for the first 3 hours, but on subsequent incubation there was a small increase. This suggested that pyruvate probably inhibited the conversion of methionine to ethylene, and the inhibitory effect was removed when pyruvate was exhausted from the medium. Even if the addition of pyruvate increased the production of 2-keto-4-thiomethylbutyrate (KMBA) from methionine through the aminotransferase reaction, the ethylene production was not stimulated. This indirectly suggested that KMBA was not an intermediate in the conversion of methionine to ethylene in Saccharomyces cerevisiae. Lieberman and

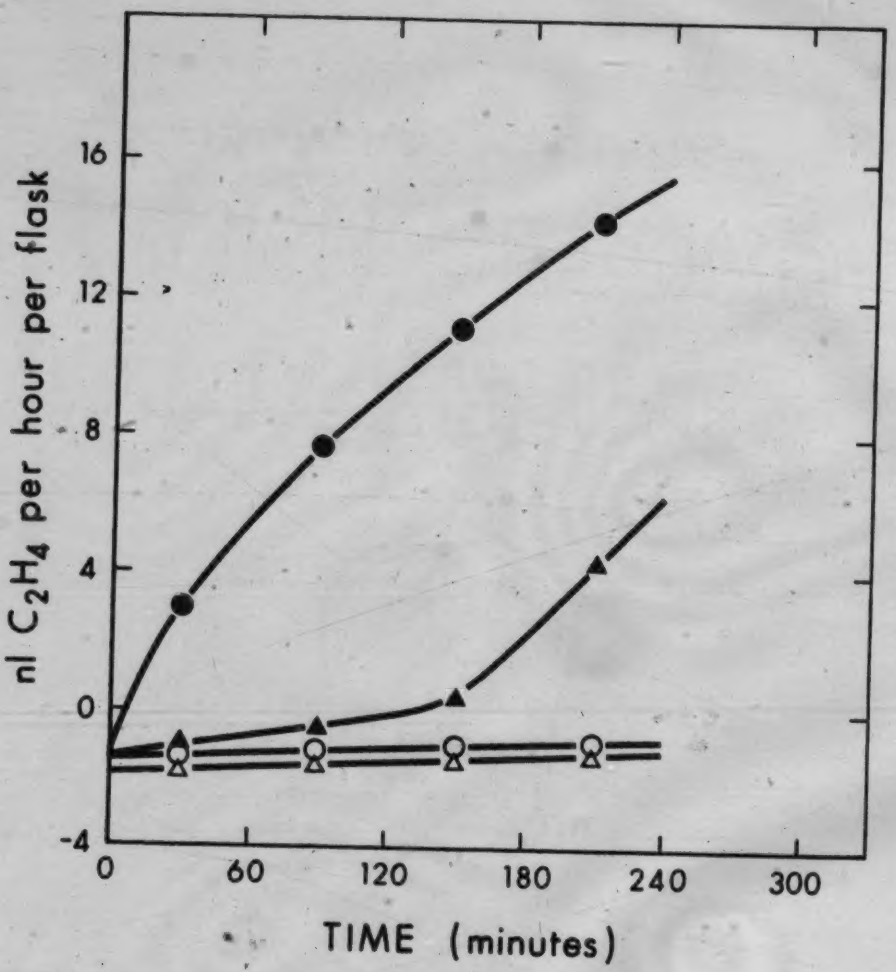
Fig. 13. Effect of pyruvate on methionine-stimulated ethylene production in *Saccharomyces cerevisiae* (X-2180-1B). 40 ml quantities of lactate grown yeast culture were used for ethylene collection. L-methionine (1 mM) and pyruvate (1 mM) were added at the beginning of ethylene collection period.

○ Blank (fresh medium)

△ Control

● 1 mM L-methionine

▲ 1 mM L-methionine + 1 mM potassium pyruvate


nl C_2H_4 per hour per flask
16
12
8
4
0
-4
0
60
120
180
240
300
TIME (minutes)

Kunishi (85) and Baur et al. (15) also came to the same conclusion from experiments in which KMBA was fed to apples and avacado.

The inhibitory effect of pyruvate on ethylene production by Saccharomyces cerevisiae (X-2180-1B) was verified in a separate experiment (Figure 14). Even at a concentration of 0.1 mM, pyruvate inhibited ethylene production from yeast filtrate by 37% and at 10 mM concentration of pyruvate this inhibition was increased to 82%. The extent of inhibition of ethylene production by the yeast in the presence of 0.1 mM and 1.0 mM pyruvate was very small, but at 10 mM concentration the inhibition reached a value of 23%. The decreased inhibition of ethylene synthesis by pyruvate in yeast may result from the capacity of yeast to utilize pyruvate, and thus remove its inhibitory effect. It is interesting to note that when the yeast is grown with lactate as the sole carbon source, it does not produce ethylene (Table 1). This lack of ethylene production may result from a large intracellular concentration of pyruvate, which is directly derived from lactate.

13. Effects of L-alanine and L-cysteine on Ethylene Production by Saccharomyces cerevisiae (X-2180-1B)

The effects of L-cysteine and L-alanine on ethylene production by yeast were studied, and the results are presented in Figure 15.

L-Alanine (1 mM) seemed to have little initial effect on ethylene production by the yeast, but 3 hours after the addition of the amino acid, the rate of ethylene production declined considerably, suggesting a possible inhibition. In the presence of L-cysteine (1 mM), the rate of ethylene production was stimulated initially, but on subsequent incubation the rate dropped at a faster rate than in the control.

The inhibitory effect of L-alanine on ethylene production by the yeast may result from it being converted to pyruvate through the

Fig. 14. Inhibition of ethylene production by pyruvate. Saccharomyces cerevisiae (X-2180-1B) was grown in glucose medium to the mid-log phase. Forty ml of the culture or filtrate were used ethylene collection. Potassium pyruvate was added at the beginning ethylene collection period.

○ Yeast plus growth medium

● Cell-free growth medium (filtrate)

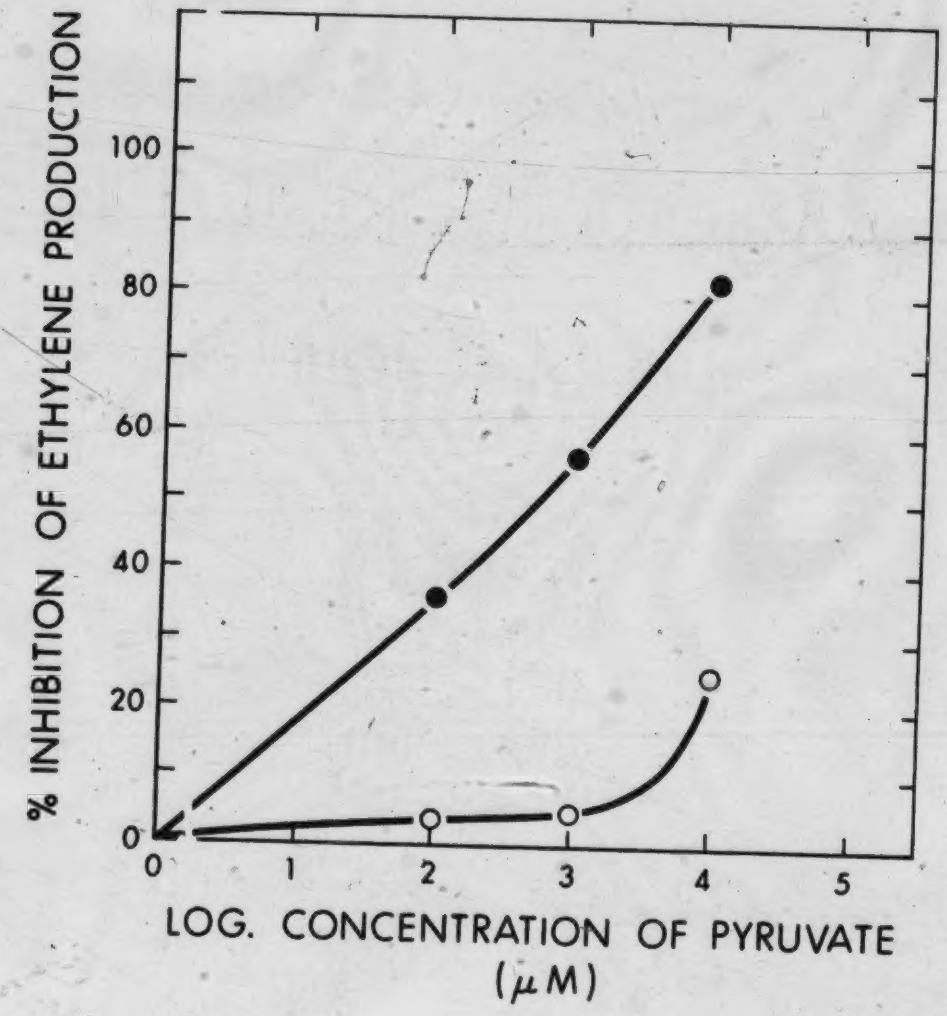
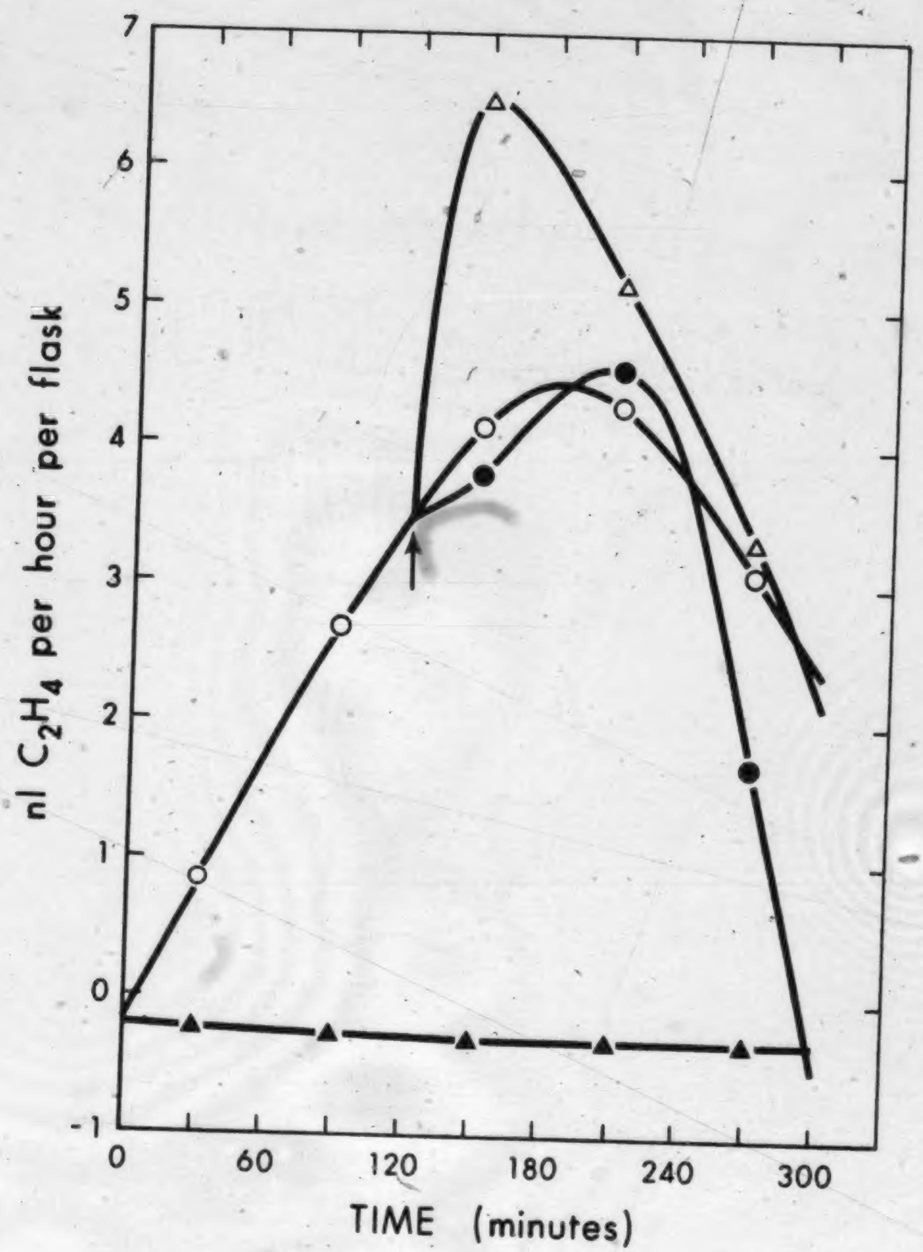

% INHIBITION OF ETHYLENE PRODUCTION
100
80
60
40
20
0
0
1
2
3
4
5
LOG. CONCENTRATION OF PYRUVATE
(μM)

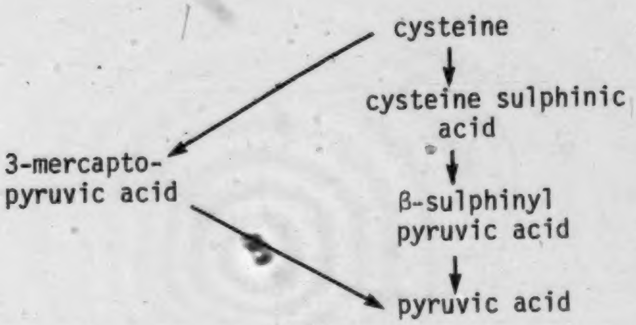
Fig. 15. Effect of L-alanine and L-cysteine on ethylene production by *Saccharomyces cerevisiae* (X-2180-1B). The yeast was grown in lactate medium to the early log-phase. Forty ml quantities of the culture were used for ethylene collection. Glucose (2%) was added at the beginning of ethylene collection period. L-Alanine and L-cysteine were added at the time indicated.

▲ Control
○ 2% glucose at zero time
● 2% glucose at zero time + 1 mM L-cysteine at 120 min
△ 2% glucose at zero time + 1mM L-cysteine at 120 min
↑ Point of addition

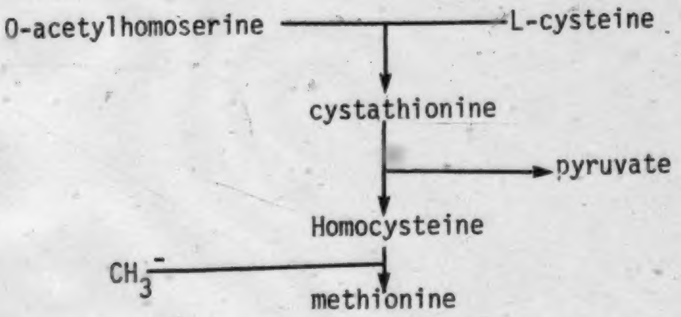

nl C_2H_4 per hour per flask
7
6
5
4
3
2
1
0
-1
0
60
120
180
240
300
TIME (minutes)

action of an amino transferase. It has already been shown that pyruvate inhibits ethylene production in the yeast (Figure 13 and 14). This observation is not in agreement with the report (136) that L-alanine stimulates ethylene production in Penicillium.

The fact that L-cysteine may inhibit ethylene production is known. Thus Lieberman et al. (86) reported that L-cysteine was a powerful inhibitor of methionine-stimulated ethylene production in apples. The inhibitory effect of L-cysteine may also be through its conversion to pyruvate. For example, catabolism of L-cysteine (shown below) yields pyruvate.



Also in yeast, one of the products of the biosynthetic pathway of methionine from O-acetylhomoserine and L-cysteine, is pyruvate (44). For each molecule of methionine synthesized, one molecule of pyruvate is also derived. Therefore, pyruvate may be the actual inhibitor of ethylene synthesis, when L-cycsteine and L-alanine are fed to the yeast.



This also indirectly suggests that if the yeast is grown in a methionine-free medium it will synthesize its own methionine with the concomitant production of pyruvate, and the pyruvate thus produced will have an inhibitory effect on the conversion of methionine to ethylene. The lack of ethylene production when the yeast was grown with $(NH_4)_2HOP_4$ as the sole source of nitrogen (Table 1) might have partly resulted from the inhibitory effect of pyruvate formed during the synthesis of methionine. On the other hand, in the presence of exogenously added methionine the synthesis of ethylene is not subject to inhibition by pyruvate produced during the synthesis of methionine, since under these conditions methionine biosynthesis is retarded through feed back inhibition (44).

14. Role of Glucose in the Stimulation of Ethylene Production

Experiments reported in earlier sections of this thesis have clearly shown that the presence of glucose is needed for maximal rate of ethylene production from methionine (Figures 7 and 8). Also it has been demonstrated that highest rate of production of ethylene occurred when the glucose concentration was low and the yeast was not repressed (Figure 3).

Mapson (108) suggested that glucose provides H_2O_2 through the action of glucose oxidase and this H_2O_2 is used by a peroxidase to form ethylene from methionine. Destruction of H_2O_2 by catalase has been shown to inhibit ethylene production from methional by cauliflower florets (113).

If H_2O_2 generated by the action of glucose oxidase on D-glucose is responsible for the stimulation of ethylene production from methionine, one should be able to demonstrate this role of glucose by removing H_2O_2

by exogenously added catalase. (Lieberman and Kunishi (85) have presented presumptive evidence that apple tissues are penetrated by catalase.) Producing H_2O_2 by adding glucose oxidase to the medium that contains D-glucose on the other hand, may stimulate ethylene production instantaneously. These aspects were studied and the results are presented in Figure 16.

Addition of glucose oxidase to the medium stimulated ethylene production as expected. But catalase also seemed to increase ethylene production by the yeast in presence of glucose and methionine. It may be recalled that Mapson and Wardale (113) also got a stimulation of ethylene production by catalase in cauliflower extracts up to 8 hours of incubation. Lieberman and Kunishi (85) provided further evidence that production of ethylene from methionine by cauliflower florets was stimulated by catalase.

Since catalase increases the ethylene production in Saccharomyces, the stimulatory action of glucose oxidase cannot result from the generation of H_2O_2. The stimulatory effect of glucose oxidase may be a result of reducing the glucose concentration from 2% to an optimum level (Figure 3).

Catalase seemed to decrease ethylene production by the yeast if the medium did not contain added methionine (Figure 17). The effect of glucose oxidase, once again, was to increase ethylene production even in the absence of added methionine, but this effect was short-lived and within 3 hours it decreased to the level of control. From this study it appears that ethylene production by the yeast in the absence of methionine (but in the presence of glucose) may be dependent on H_2O_2, but in presence of added methionine H_2O_2 generated by glucose oxidase does not seem to be essential. This study, therefore, does not support the view (108) that

Fig. 16. Effect of catalase and glucose oxidase on ethylene production by *Saccharomyces cerevisiae* (X-2180-1B). The yeast was grown in lactate medium to the mid-log phase. Forty ml quantities of the culture were used for ethylene collection. Glucose(2%) was added at the beginning of the collection period. L-Methionine, catalase and glucose oxidase were added at the time indicated.

○ Control

△ 1 mM L-methionine + 10 mg catalase

● 1 mM L-methionine

▲ 10 mg glucose oxidase + 1 mM L-methionine

↓ Point of addition

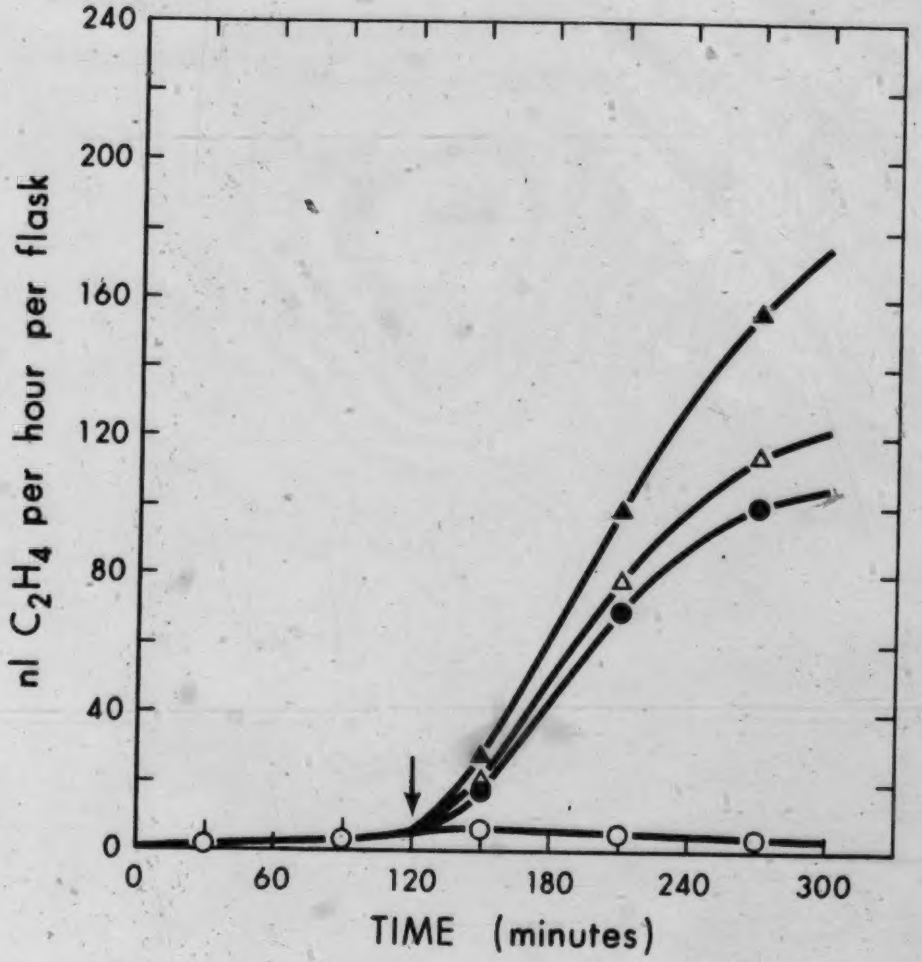
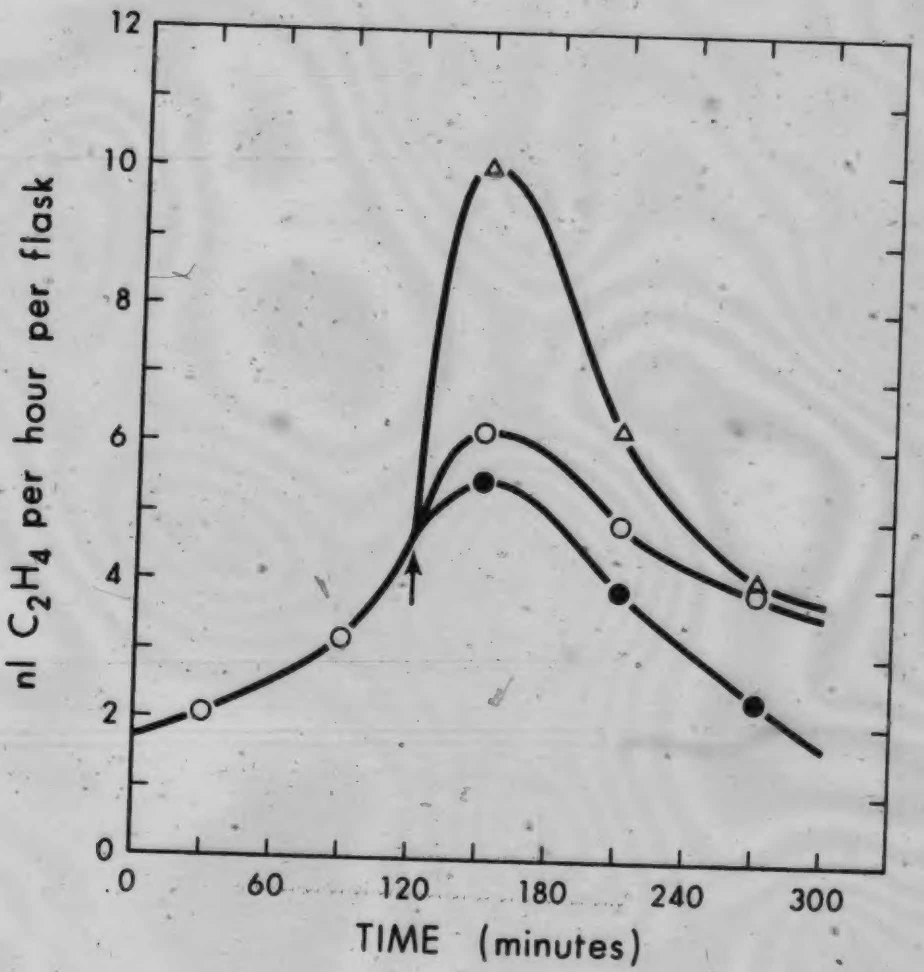

nl C_2H_4 per hour per flask
0
40
80
120
160
200
240
0
60
120
180
240
300
TIME (minutes)

Fig. 17. Effect of catalase and glucose oxidase on ethylene production by Saccharomyces cerevisiae (X-2180-1B) in the absence of added methionine. The yeast was grown in lactate medium to the mid-log phase. Forty ml quantities of the culture were used for ethylene collection. Glucose (2%) was added at the beginning of the collection period. Catalase and glucose oxidase were added at the time indicated.

○ Control
● 10 mg catalase
△ 10 mg glucose oxidase
↑ point of addition of enzymes


nl C_2H_4 per hour per flask
12
10
8
6
4
2
0
0
60
120
180
240
300
TIME (minutes)

the role of glucose in ethylene biosynthesis is to provide H_2O_2.

Direct addition of H_2O_2, however, had a transient stimulatory effect on ethylene production by S. cerevisiae (Table 6). But this stimulatory effect of H_2O_2 on ethylene production was less than that of glucose.

Addition of glucose to the yeast growing in lactate medium caused an immediate drop in the intracellular concentration of free L-alanine (Table 7). Since exogenously supplied L-alanine has been shown to inhibit ethylene production (Fig. 15) the stimulatory effect of glucose on ethylene production by S. cerevisiae may be through lowering the intracellular concentration of L-alanine.

15. Effect of β-alanine on Ethylene Production by Saccharomyces cerevisiae (X-2180-1B)

β-alanine has been suggested as a precursor of ethylene in plants (118, 169, 177). Whether β-alanine could stimulate ethylene production by yeast was studied; the results are presented in Figure 18.

The extent of stimulation of ethylene production in presence of β-alanine (1 mM) was very small. At the peak of the ethylene production the stimulation amounted to only 18%. This was in contrast to the methionine-stimulated ethylene production--an increase of 130-fold over the control (only part of the methionine stimulated ethylene production is shown in Fig. 18). Therefore, this study does not provide evidence in support of β-alanine being a precursor of ethylene in Saccharomyces cerevisiae. β-Alanine, although not utilizable as a source of nitrogen, is a growth factor, and when added in small amounts, has been shown to increase the growth of Saccharomyces cerevisiae in the

TABLE 6. Effect of various additives on ethylene production by *Saccharomyces cerevisiae* (X-2180-1B)

Additives	nl ethylene/hr		
	0-60 (min)	60-120 (min)	120-180 (min)
1 mM L-methionine	5.3	4.6	5.6
2% glucose	3.0	4.5	5.3
1 mM L-methionine + 2% glucose	10.0	15.0	48.0
1 mM L-methionine + 2% glucose + 10 glucose oxidase	7.5	21.0	68.0
1 mM L-methionine + 2% glucose + 10 mg glucose oxidase + 10 mg catalase	7.0	18.0	63.0
1 mM L-methionine + 1 mM H_2O_2	8.0	5.3	4.5

The yeast was grown in lactate medium to the mid-log phase. Forty ml quantities of the culture (60 mg dry wt) were used for ethylene collection. Various additions were made at the beginning of the collection period.

TABLE 7. Effect of carbon source on the intracellular levels of free L-alanine in Saccharomyces cerevisiae (X-2180-1B)

Carbon source	micromoles L-alanine/g yeast (dry wt)
DL-Lactate	80
D-Glucose	10
DL-Lactate + D-Glucose	13

The yeast was grown in lactate or glucose medium to the mid-log phase and the L-alanine content of the cells was determined. In the third case the yeast was grown in lactate medium to the early log-phase and 2% glucose was added to the culture . After 2 hours the alanine content in the cells was determined.

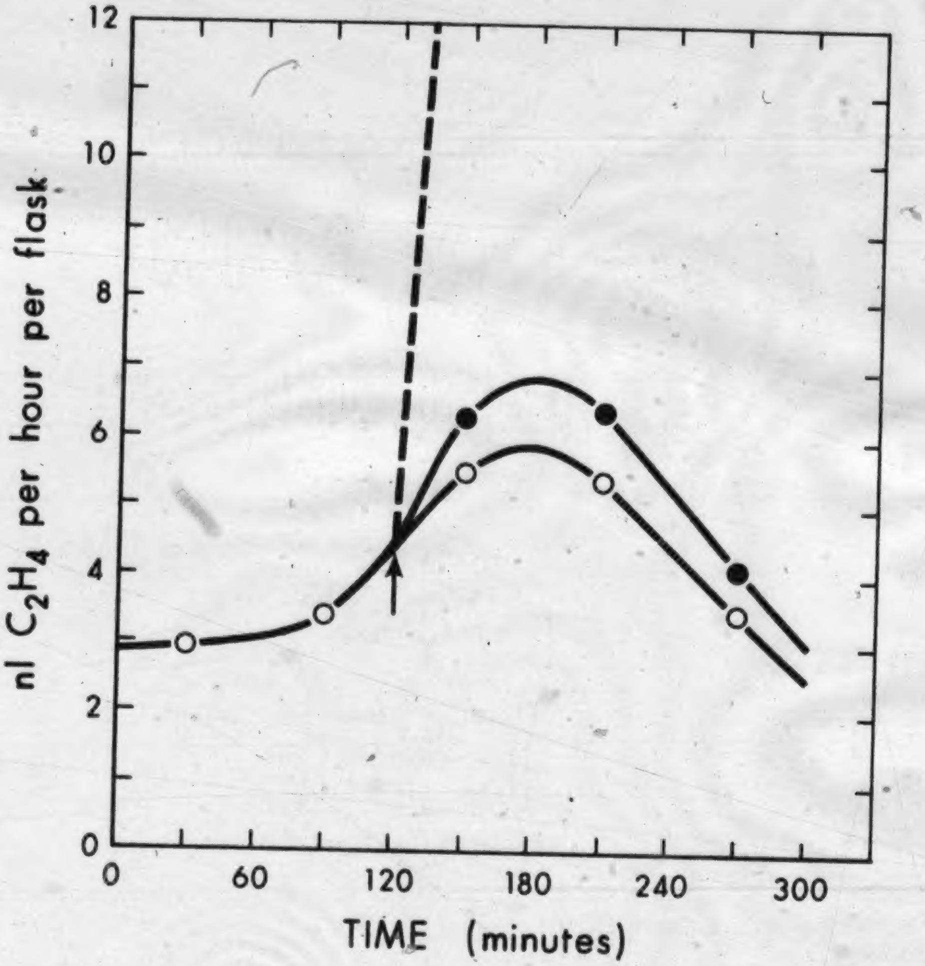
Fig. 18. Effect of β-alanine on ethylene production by *Saccharomyces cerevisiae* (X-2180-1B). The yeast was grown in lactate medium to the mid-log phase. 40 ml quantities of the culture were used for ethylene collection. Glucose (2%) was added 15 minutes prior to the start of ethylene collection. β-alanine and L-methionine were added at the time indicated.

○ Control

● 1 mM β-alanine

— — — — 1 mM L-methionine

↑ Point of addition


nl C_2H_4 per hour per flask
12
10
8
6
4
2
0
0
60
120
180
240
300
TIME (minutes)

presence of a source of nitrogen (174). Therefore, the slight stimulation of ethylene production in presence of β-alanine might result from increased growth of the yeast. The rate of ethylene production by the yeast from L-methionine (1 mM) was not altered by β-alanine (not included in Figure 18).

16. Production of Ethylene by Adenine- and Methionine-Requiring Mutant Saccharomyces cerevisiae

A mutant yeast (*Saccharomyces* *cerevisiae* G1332) was used in this study. This mutant lacked the capacity to synthesize adenine and methionine and an exogenous supply of these compounds was needed for growth. Choice of this mutant for the study of ethylene biosynthesis was based on the following reasons:

a. Methionine has been shown to stimulate ethylene production in yeast. But direct proof for its conversion to ethylene in yeast is lacking, although this has been established in plants by tracer studies. The use of a met$^-$ mutant yeast might provide an answer as to the role of methionine in the stimulation of ethylene production in yeast. If methionine is the precursor of ethylene in yeast, then the met$^-$ mutant would not produce any ethylene in the absence of exogenously supplied methionine. On the other hand, ethylene production by the mutant should be stimulated by an exogenous supply of methionine.

b. It may still be argued that by depriving the mutant yeast of an exogenous source of methionine, its growth will be inhibited and the arrested growth may be the actual cause for the lack of ethylene production, rather than non-availability of methionine as precursor for ethylene rpoduction. By using a double mutant this difficulty can be circumvented. The mutant does not grow even in the presence of added methionine if adenine is omitted from the medium. Therefore, if ethylene production by the mutant can be demonstrated in the presence of methionine, but in the absence of adenine (but not _vice versa_), it may be taken as added proof in support of methionine being a precursor of ethylene in yeast.

The mutant _S. cerevisiae_ (G1332) was grown at 27° for 20 hours in complete medium as described in Materials and Methods. The cells were harvested by filtering through a Millipore membrane filter (0.8 μ pore diameter), and washed 4 times with fresh medium that did not contain methionine or adenine. The cells were suspended in the same medium, and the effects of adenine and methionine on ethylene production were determined. The results are presented in Figure 19.

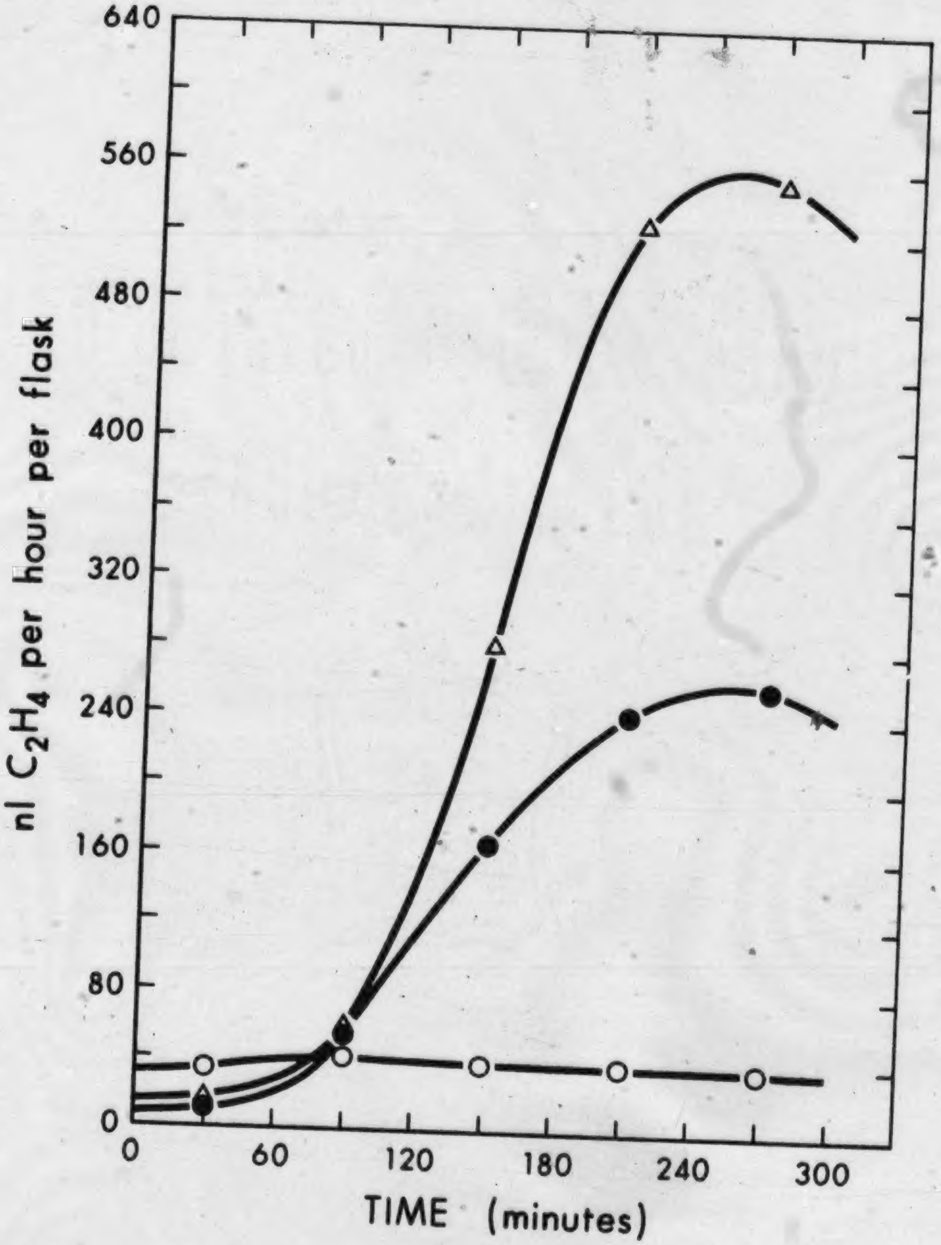
In the absence of added methionine and adenine the amount of ethylene produced by the mutant was small, and the rate of production was constant. When L-methionine (1 mM) was added to the suspension, the rate of production of ethylene dropped slightly at the beginning, but then increased rapidly; an increase of 6.5-fold over the control. Addition of L-methionine and adenine showed a similar trend in the beginning, but subsequent stimulation of ethylene production was considerably higher in this case (15-fold increase over the control) than in the sample that contained L-methionine but no adenine. This result shows that the mutant yeast, which cannot synthesize its own methionine, does not produce any appreciable amount of ethylene unless the amino acid is exogenously supplied. Therefore, it may be tentatively concluded that methionine may be the precursor of ethylene in yeast.

Fig. 19. Production of ethylene by adenine- and methionine-requiring *Saccharomyces cerevisiae* G1332. The yeast was grown in complete medium as described in Materials and Methods. The cells were washed and suspended in media containing adenine, methionine or adenine + methionine. In each case 40 ml cell suspension contained 125 mg yeast (dry wt)

○ + adenine (1mM)

● + L-methionine (1mM)

△ + adenine (1mM) + L-methionine (1mM)



nl C_2H_4 per hour per flask
640
560
480
400
320
240
160
80
0
0
60
120
180
240
300
TIME (minutes)

It appeared that even in washed cells enough endogenous methionine and adenine were available to sustain the growth of the mutant yeast for a few hours. Initially each flask contained 125 mg (dry weight) of yeast. After 5 hours the dry weights were 188, 196 and 202 mg in flasks containing methionine, adenine and methionine + adenine, respectively. Therefore, the basal level of ethylene production observed in the absence of exogenously added methionine may result from the endogenous methionine.

17. Production of Ethylene from L-methionine-U-^{14}C

Results presented in Table 8 indicate that radioactivity from L-methionine-U-^{14}C was incorporated into ethylene by *S. cerevisiae* G1332. The yeast produced 13.4 nmoles of ethylene during 3 hours. The specific activity of the ethylene was 248 dpm/natoms of carbon. This specific activity accounted for 48% conversion efficiency.

Considering the short duration of the experiment (3 hours), in the present study, the rate of conversion of methionine to ethylene seems quite efficient. In this respect it is interesting to note that with a collection period of 24 hours Jacobsen and Wang (68) got only 5% conversion efficiency when DL-methionine-U-^{14}C was fed to *Penicillium*.

18. The Effect of N-formylmethionine and Methionine-Containing Dipeptides on Ethylene Production by Methionine-Requiring Saccharomyces cerevisiae

Demorest and Stahman (46) reported that in a model system, N-formylmethionine and peptides with C-terminal methionine yielded more ethylene than methionine itself. Naider *et al* (123) observed that a methionine-requiring auxotroph of *Saccharomyces cerevisiae* could utilize methionine-containing peptides for growth, if the C-terminal residue of the peptide was methionine. Peptides with other amino acids in the

TABLE 8: Production of Radioactive Ethylene from L-Methionine-U-^{14}C by Saccharomyces cerevisiae G1332

Total ethylene /3 hr (nmoles)	Total activity of ethylene (dpm)	sp.activity of ethylene dpm/natom of carbon	sp. activity of L-methionine dpm/natom of carbon	%conversion
13.4	6646	248	511	48

The mutant yeast was grown in complete medium to the mid-log phase. Cells were harvested by filtration, washed with methionine-free fresh medium and suspended in the same medium. Forty ml of the suspension was used for the experiment. Forty micromoles of L-methionine-U-^{14}C(sp. activity 1.15 microCurie/micromole) were added. Ethylene was collected for 3 hours and the radioactivity determined as described in Materials and Methods. Forty ml of the suspension contained 60 mg yeast (dry wt).

C-terminal position did not support growth. The authors concluded that the C-terminal methionine residue might somehow be involved in the specificity of the peptide transport system.

The effect of N-formylmethionine and methionine containing peptides on ethylene production by a methionine-requiring auxotroph Saccharomyces cerevisiae (G1332) was studied. The yeast was grown in complete medium at 27° for 20 hours as described in Materials and Methods.

The initial concentrations of dipeptides were adjusted so that on their hydrolysis the concentration of L-methionine would be 1 mM. The results are presented in Figure 20 and Table 9.

The mutant yeast produced only very small amounts of ethylene when the medium did not contain L-methionine or a derivative of L-methionine. N-formylmethionine increased ethylene production nearly 70-fold over the control within one hour of its addition to the yeast suspension, but thereafter the rate of production of ethylene leveled off. Although N-formylmethionine increased the ethylene production, it did not stimulate the growth of the yeast (Table 9).

L-Methionine and methionine-containing peptides stimulated both growth and ethylene production. But with these compounds there was a short lag before ethylene synthesis was stimulated. The rate of ethylene production in presence of L-methionine increased at a rapid rate after the initial lag and two hours after the addition of the amino acid the amount of the ethylene produced was 200 times more than that produced by the control. Methionylglycine also stimulated ethylene production by the yeast, although peptides with amino acid residues other than methionine in terminal position were reported to be ineffective in yielding ethylene in model systems (46). Naider et al. (123) suggested that peptides with

Fig. 20. Effect of L-methionine, N-formylmethionine, L-methionylglycine and L-methionylmethionine on ethylene production by Saccharomyces cerevisiae G1332. The yeast was grown in complete medium as described in Materials and Methods. The cells were washed and suspended in fresh growth medium that contained adenine but no methionine. Forty ml quantities of the suspension were used for ethylene collection. Various additions were made at the beginning of the collection period. Forty ml suspension contained 75 mg yeast (dry wt)

○ Control

● N-formylmethionine (1mM)

▲ L-methionine (1mM)

△ L-methionylglycine (1mM)

□ L-methionylmethionine(0.5mM)

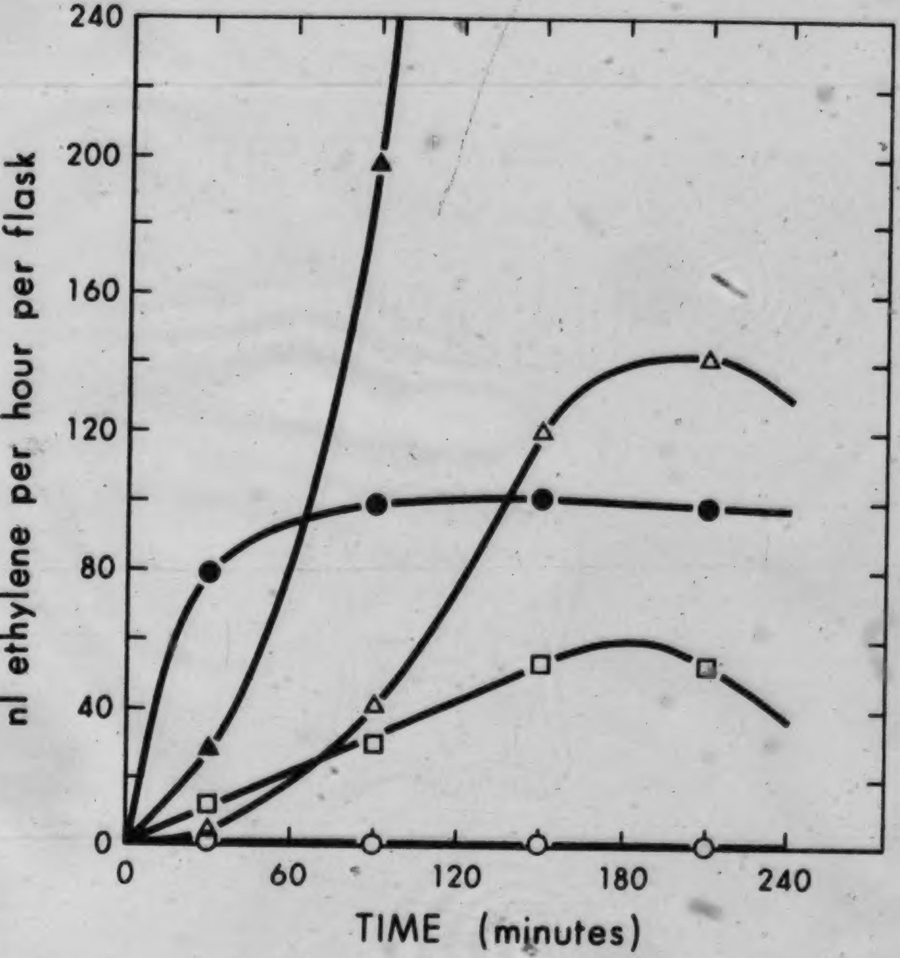

240
200
160
120
80
40
0
0
60
120
180
240
nl ethylene per hour per flask
TIME (minutes)

TABLE 9: Effect of L-methionine, N-formyl-L-methionine and Methionine-Containing Peptides on Ethylene Production by Methionine- and Adenine-Requiring *Saccharomyces cerevisiae* G1332

Treatment	Cell Mass (dry wt) at 0 min (mg)	Cell Mass (dry wt) at 240 min (mg)	Ethylene Production nℓ/4 hr	Ethylene Production Increase (fold)
Control	75	110	5	1
+ L-methionine (1 mM)	75	134	1841	368
+ N-formyl-L-methionine (1 mM)	75	107	378	76
+ L-methionyl glycine (1 mM)	75	136	304	61
+ L-methionylmethionine (0.5 mM)	75	131	148	30

The mutant yeast was grown in complete medium at 27° as described in Materials and Methods. Mid-log phase cells were obtained by filtration. The cells were washed with fresh methionine-free medium, and suspended in the same medium. Forty ml quantities were distributed in collection flask. The additions were made at the beginning of the collection period.

amino acid residues other than methionine in the C-terminal position are not transported into the methionine-requiring auxotrophic yeast. Present studies indicated that L-methionylglycine not only stimulated ethylene production but also supported the growth of the mutant yeast (Table 9). Therefore, it seems that either L-methionylglycine is transported into the yeast or it is hydrolyzed by an extracellular peptidase to L-methionine and glycine, before being transported.

Methionylmethionine also stimulated ethylene production by the yeast, but this stimulation was less than that observed in the presence of L-methionylglycine.

Maximum ethylene production by the yeast was observed in the presence of L-methionine, followed by N-formylmethionine, L-methionylglycine and methionlmethionine, respectively. But in model systems N-formylmethionine and peptides with C-terminal methionine residues yielded more ethylene than methionine itself (46). In yeast, slow uptake of the derivatives of methionine may be the rate limiting factor rather than low rate of conversion of these derivatives to ethylene.

19. Production of Ethylene from Methional

Relatively large amounts of ethylene were formed when methional was added to uninoculated lactate medium (Table 10). Addition of peroxidase to the medium containing methional decreased the ethylene production by 26%. This indicated that peroxidase may not be involved in the conversion of methional to ethylene, but peroxides are probably necessary. Production of ethylene from methional was increased about 14 times by the addition of glucose + glucose oxidase. Peroxidase seemed to enhance the ethylene production stimulated by glucose + glucose oxidase.

TABLE 10: Production of Ethylene from Methional

Sample	nl ethylene/hr
Fresh medium	2
Fresh medium + 1mM Methional	116
Fresh medium + 1mM Methional + 1mg Peroxidase	88
Fresh medium + 2% glucose + 10mg glucose oxidase	4
Fresh medium + 2% glucose + 10mg glucose oxidase + 1mM methional	1544
Fresh medium + 2% glucose + 10mg glucose oxidase + 1mM methional + 1mg peroxidase	1731

Forty ml of fresh lactate medium was used in each case. Additions were made at the beginning of the ethylene collection. The activities of the enzymes as reported by the manufacturer (Sigma Chemical Co.) were as follows.

Glucose oxidase : 18.9 units/mg
horseradish peroxidase : 72 units/mg

The mechanism by which glucose + glucose oxidase stimulates ethylene production from methional is not clearly understood. The stimulation may be mediated through the production of H_2O_2 as suggested by Mapson (108). Since large amounts of ethylene from methional were produced in uninoculated medium, the suitability of this compound as a precursor of ethylene in yeast was not tested.

B. EFFECTS OF ETHYLENE ON YEAST

1. Ethylene and Respiration of Yeast

a. Respiratory activity of yeast grown in the Presence of ethylene

Saccharomyces cerevisiae (X-2180-1B) was grown at 24-26° in lactate medium for 16 hours with sterile air or 100 ppm ethylene in air as described in Materials and Methods. The cells were harvested by centrifugation, washed twice with distilled water and suspended in 0.1 M potassium phosphate buffer pH 5.0. The rate of O_2 uptake was determined with 0.5% glucose as substrate by Warburg method at 25°. The results are presented in Figure 21.

The yeast grown in the presence of 100 ppm ethylene (hereafter referred to as ethylene-grown yeast) took up 33% less oxygen during a period of 2 hours than the air-grown yeast (Figure 21). The low uptake of oxygen by the ethylene-grown yeast may result from a low rate of glycolysis caused by decreased availability of ATP for glucose phosphorylation. The amount of ATP present in ethylene-grown yeast was found to be significantly lower than that found in air-grown yeast (Table 11).

APTase may play a role in the regulation of intracellular concentration of ATP and the glycolytic flux. Racker (141) suggested that excess of ATPase would inhibit glycolysis because ATP required for glucose phosphorylation would become limiting. Phillips (138) and Olson and Spencer (128, 129) reported that the ATPase activity of isolated pea mitochondria was stimulated by 100 ppm ethylene. Malhotra and Spencer (105) showed that isolated pea mitochondrial ATPase itself was stimulated by 100 ppm ethylene in the presence of certain concentrations of carbon

Fig.21. Effect of growing Saccharomyces cerevisiae (X2180-1B) in presence of 100 ppm ethylene in air on respiration. The yeast was grown in lactate medium in presence or absence of 100 ppm ethylene in air. Cells were washed and the respiration determined in 0.1M potassium phosphate buffer (pH 5) with 0.5% glucose as substrate.

○ Yeast grown in absence of applied ethylene

● Yeast grown in the presence of 100 ppm ethylene in air

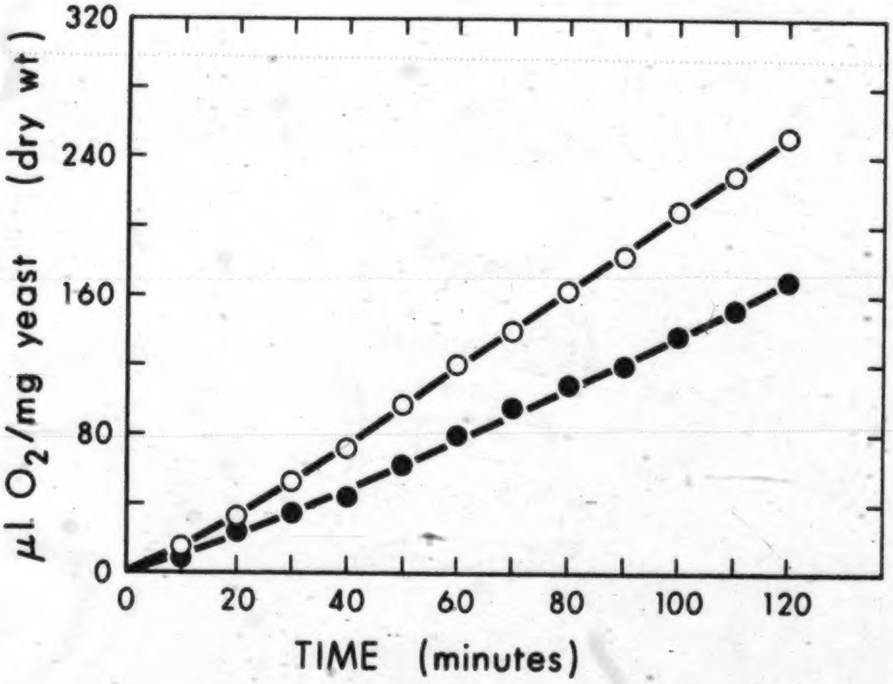

320
240
160
80
0
0
20
40
60
80
100
120
μl. O₂/mg yeast (dry wt)
TIME (minutes)

TABLE 11. Intracellular Levels of ATP and ADP in Saccharomyces cerevisiae (X-2180-1B) Grown in Lactate Medium in the Presence and Absence of Added Ethylene

Treatment	nmoles per ml Culture / A_{650} ATP	nmoles per ml Culture / A_{650} ADP	ATP/ADP
Air	8.2	145.7	0.056
100 ppm ethylene in air	5.2	93.5	0.056

The yeast was grown at 27° in lactate medium. Mid-log phase cells were used for the estimation of ATP and ADP. Cells obtained from 5-10 ml culture were extracted with 5 N perchloric acid. The extract was neutralized to pH 7.4 with buffered KOH. Potassium perchlorate removed by centrifugation. Aliquots of supernatant layer were used for the determination of ATP and ADP by the Method described by Maitra and Estabrook (102). The results are average values of triplicate determinations.

dioxide. The low oxygen uptake by ethylene-grown yeast may result from decreased amounts of ATP available for glucose phosphorylation, and increased ATPase activity may partially be responsible for this decrease.

b. Respiration of the yeast starved in presence of ethylene

The endogenous respiration (respiration in the absence of any added substrate) of the lactate-grown yeast decreased with increasing starvation period (Figure 22). But the endogenous respiration of the yeast starved in the presence of 100 ppm ethylene in air did not decrease as fast as the respiration of the yeast starved in the presence of air. This indirectly suggests that starving the yeast in presence of 100 ppm ethylene in air results in an increased mobilization of endogenous substrates required for respiration.

Exogenously applied ethylene is known to increase respiration of mature fruits and vegetative tissues (21, 146). The ethylene-induced respiration has been shown to differ qualitatively from normal respiration. The former is reported to be more cyanide resistant than the latter (161). In the present study, the endogenous respiration of the yeast starved in air or in 100 ppm ethylene in air (100 ml per min), was found to be totally resistant to 10^{-4} M KCN. Stickland (172, 173) observed that the endogenous respiration of baker's yeast was more resistant to cyanide than the respiration in the presence of added glucose. He found that endogenous respiration was inhibited only 51% by 10^{-4} M KCN, whereas in presence of glucose the inhibition amounted to 100%.

The respiration in the presence of added glucose of previously starved yeast followed a different pattern (Figure 23). The rate of oxygen uptake depended on the concentration of glucose in the assay medium. The amount of oxygen taken up by the starved yeast (starved for 90 min

Fig. 22. Effect of starving Saccharomyces cerevisiae (X-2180-1B) in the presence of 100 ppm ethylene in air on the rate of oxygen uptake. Mid-log phase cells obtained by growing the yeast in lactate medium were used.

● Starved in the presence of 100 ppm ethylene in air

○ Starved in the presence of air

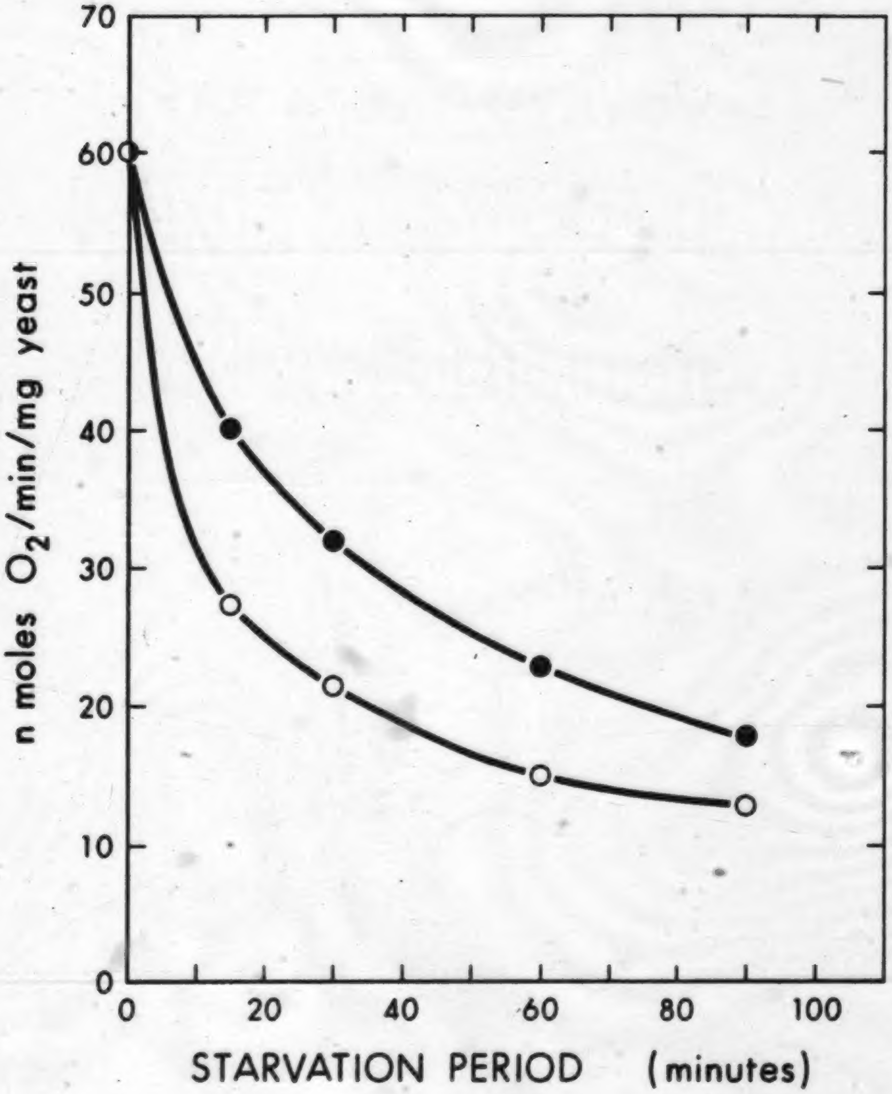

n moles O_2/min/mg yeast
70
60
50
40
30
20
10
0
0
20
40
60
80
100
STARVATION PERIOD (minutes)

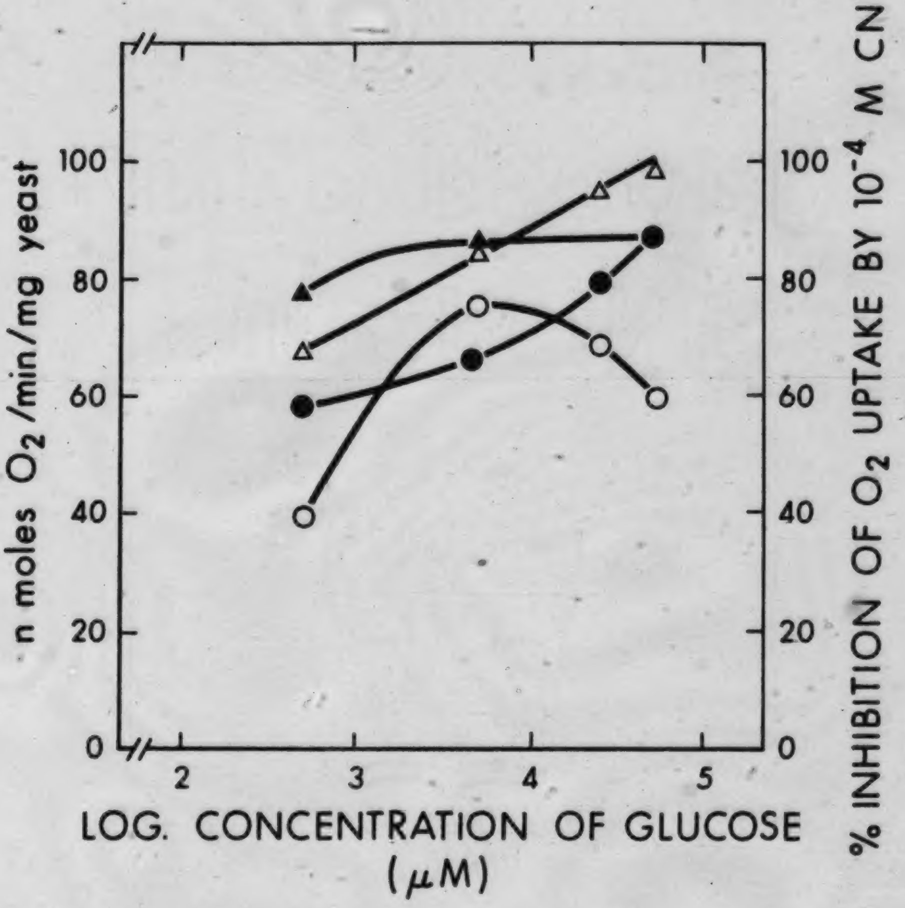
Fig. 23. Effect of glucose concentration on the respiration of previously starved Saccharomyces cerevisiae (X-2180-1B) Mid-log phase cells obtained by growning the yeast in lactate medium were starved in 0.1 M potassium phosphate buffer (pH 5.0) in the presence of 100 ppm ethylene in air or air for 90 min. Respiration was determined by the oxygraph method in presence various concentrations of glucose and glucose + 10^{-4}M KCN.

○ Starved in the absence of ethylene

● Starved in the presence of ethylene

△ % of inhibition by 10^{-4} M KCN, starved in the absence of ethylene

▲ % of inhibition by 10^{-4} M KCN, starved in the presence of 100 ppm ethylene in air


n moles O_2 /min/mg yeast
0
20
40
60
80
100
% INHIBITION OF O_2 UPTAKE BY 10^{-4} M CN^-
2
3
4
5
LOG. CONCENTRATION OF GLUCOSE
(μM)

with continuous bubbling of air through the cell suspension) increased rapidly with increasing glucose concentration up to 5 mM. Increasing the glucose concentration further resulted in reduced oxygen uptake. The decreased respiration at higher concentration of glucose may be a manifestation of the Crabtree effect.

The rate of oxygen uptake by the yeast starved in the presence of 100 ppm ethylene in air, on the other hand, continued to increase with increasing concentration of glucose in the assay medium. Lack of a Crabtree effect in ethylene-treated cells may indicate that either glycolysis and respiration is not under strict cellular control, or that the amount of glucose taken up by the ethylene-treated cells is not high enough to cause a Crabtree effect. There is some supporting evidence that glucose uptake is reduced by treating the yeast cells with ethylene. This aspect will be covered in a separate section.

It was reported in the previous section that the endogenous respiration of the starved yeast was resistant to 10^{-4}M KCN. But on addition of 0.5 mM glucose to the yeast starved in the absence of ethylene, the respiration was inhibited by 63.5% at a concentration of 10^{-4}M KCN. The fraction of respiration that was inhibited by this concentration of cyanide increased with increasing concentration of glucose. At a 50 mM concentration of glucose the respiration was completely inhibited by 10^{-4}M KCN.

The percentage of inhibition of respiration of ethylene-treated cells by cyanide did not increase at the same rate with increasing glucose concentration in the assay medium as did air-treated samples. The inhibition of oxygen uptake by 10^{-4}M KCN remained at 83% even though the glucose concentration in the assay medium was varied from 5 mM to 50 mM. This lack of complete inhibition of oxygen uptake by cyanide (10^{-4}M) even at a glucose concentration of 50 mM, may reflect, once again, reduced glucose uptake by the ethylene treated yeast.

2. Effects of Ethylene on Glucose Uptake and Ethanol Production by Saccharomyces cerevisiae (X-2180-1B)

The carbon source in the growth medium was 5% glucose. The yeast was grown at 27° with air or 100 ppm ethylene in air bubbling through the medium at the rate of 100 ml per minute. The amounts of glucose and ethanol in the medium at various intervals were determined by enzymatic methods as described in Materials and Methods. The results are presented in Figure 24.

There was no difference between air-treated and ethylene-treated samples in glucose uptake or ethanol production. There are two possibilities for this lack of difference between the two treatments. (a) 100 ppm ethylene in air has no effect on glucose uptake or ethanol production. (b) The control (air-treated sample) produces enough ethylene to cause the same physiological changes as in ethylene-treated samples and therefore there are no differences detectable in glucose uptake or ethanol production between air-treated and ethylene-treated samples. The second possibility seems to apply in the present case, since it has already been shown (Section A) that the yeast produces large amounts of ethylene when grown in a glucose medium.

3. Ethanol Production from Glucose by Yeast Starved in the Presence of Ethylene

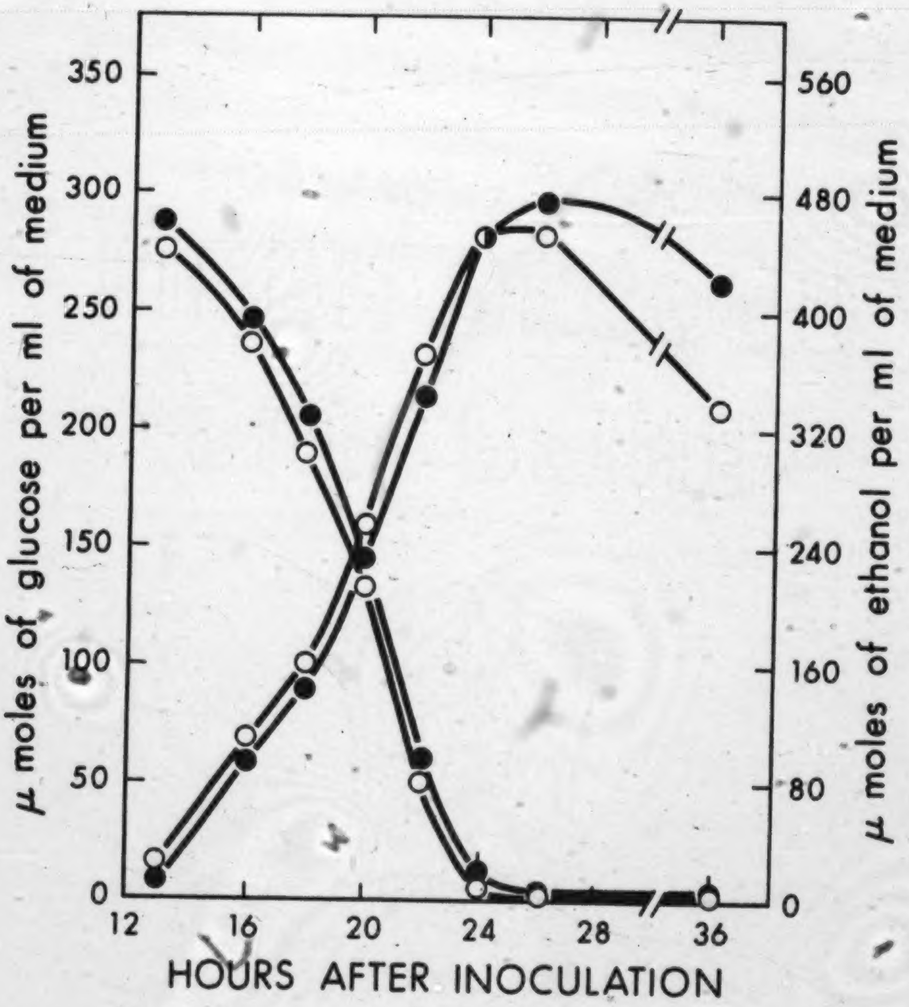
Log-phase cells obtained by growing S. cerevisiae (X-2180-1B) were suspended in 0.1M potassium phosphate buffer (pH 5.0) and were were starved for 24 hours with continuous bubbling of air or 100 ppm

Fig. 24. Glucose uptake and ethanol production by Saccharomyces cerevisiae (X-2180-1B) growing in glucose medium in presence and absence of applied ethylene. The growth medium initially contained 5% glucose.

○ Grown in the absence of applied ethylene

● Grown in the presence of 100 ppm ethylene in air

(the rising curves represent ethanol)


350
300
250
200
150
100
50
0
μ moles of glucose per ml of medium
560
480
400
320
240
160
80
0
μ moles of ethanol per ml of medium
12
16
20
24
28
36
HOURS AFTER INOCULATION

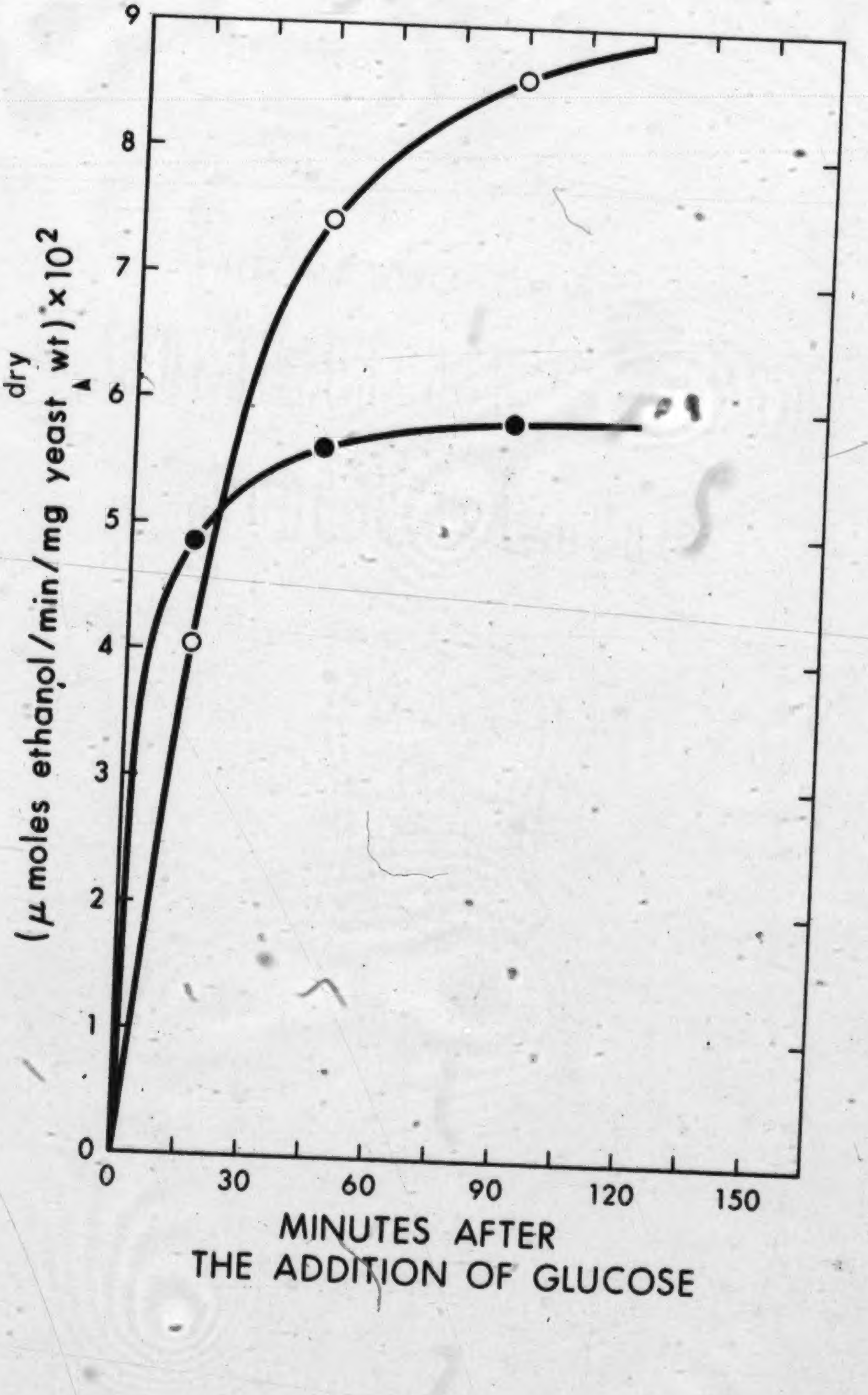
ethylene in air through the cell suspension at the rate of 100 ml per min. Glucose dissolved in 0.1 M potassium phosphate buffer (pH 5.0) was added to the cell suspension at the end of 24 hours to give a final concentration of 2%. The amount of ethanol in the suspending medium at various intervals after the addition of glucose was determined enzymatically as described in Materials and Methods. The results are presented in Figure 25.

The rate of production of ethanol was faster in ethylene-treated samples during the first 30 minutes than in air-treated samples. On further incubation the rate of production of ethanol continued to increase and reached a steady value by 2 hours. Starving the yeast in the absence of ethylene, on the other hand, resulted in a continued increase in the rate of production of ethanol from glucose. The amount of ethanol produced by ethylene-treated yeast 2 hours after the addition of glucose was 32% less than that produced by the yeast starved in the absence of ethylene. Therefore, the net effect of starving the yeast in presence of 100 ppm ethylene in air was to decrease the amount of ethanol produced from glucose, although the initial rate of production of ethanol by the ethylene-treated yeast was faster than that by the air-treated yeast. Sarkar (154) found that treatment of carrots with 100 and 2000 ppm ethylene in air resulted in decreased ethanol production. This decrease in ethanol production, he suggested, was a result of pyruvate being increasingly converted to acetate which in turn was used for respiration or synthesis of other products.

Fig. 25. Ethanol production by previously starved _Saccharomyces_ _cerevisiae_ (X-2180-1B) from glucose. Mid-log phase cells obtained by growing the yeast in lactate medium were starved for 24 hours in 0.1 M potassium phosphate buffer (pH 5.0) in the presence or absence of 100 ppm ethylene in air. 2% glucose was added and the rate of ethanol production determined.

○ Starved in the absence of applied ethylene

● Starved in the presence of 100 ppm ethylene in air


(μ moles ethanol/min/mg yeast dry wt) × 10²
0
1
2
3
4
5
6
7
8
9
0
30
60
90
120
150
MINUTES AFTER
THE ADDITION OF GLUCOSE

4. Effects of Metabolic CO_2 and Exogenously Applied Ethylene on Ethanol Production from Glucose by *Saccharomyces cerevisiae* (X-2180-1B)

When yeast is grown in a glucose medium large amounts of CO_2 are produced by fermentation and respiration. The metabolic CO_2 of yeast may offset the effects of applied ethylene since carbon dioxide is known to retard or remove many of the physiological effects of ethylene. The effect of metabolic CO_2 on ethanol production from glucose by yeast was studied. The results are presented in Figure 26.

Controls (no ethylene treatment) produced more ethanol than samples treated with ethylene. When metabolic CO_2 was absorbed by alkali, the total amount of ethanol produced by the yeast in the absence of applied ethylene was 47 μmoles/ml. The corresponding values for samples treated with 100 ppm and 2000 ppm ethylene in air were 34 and 26 μmoles/ml, respectively.

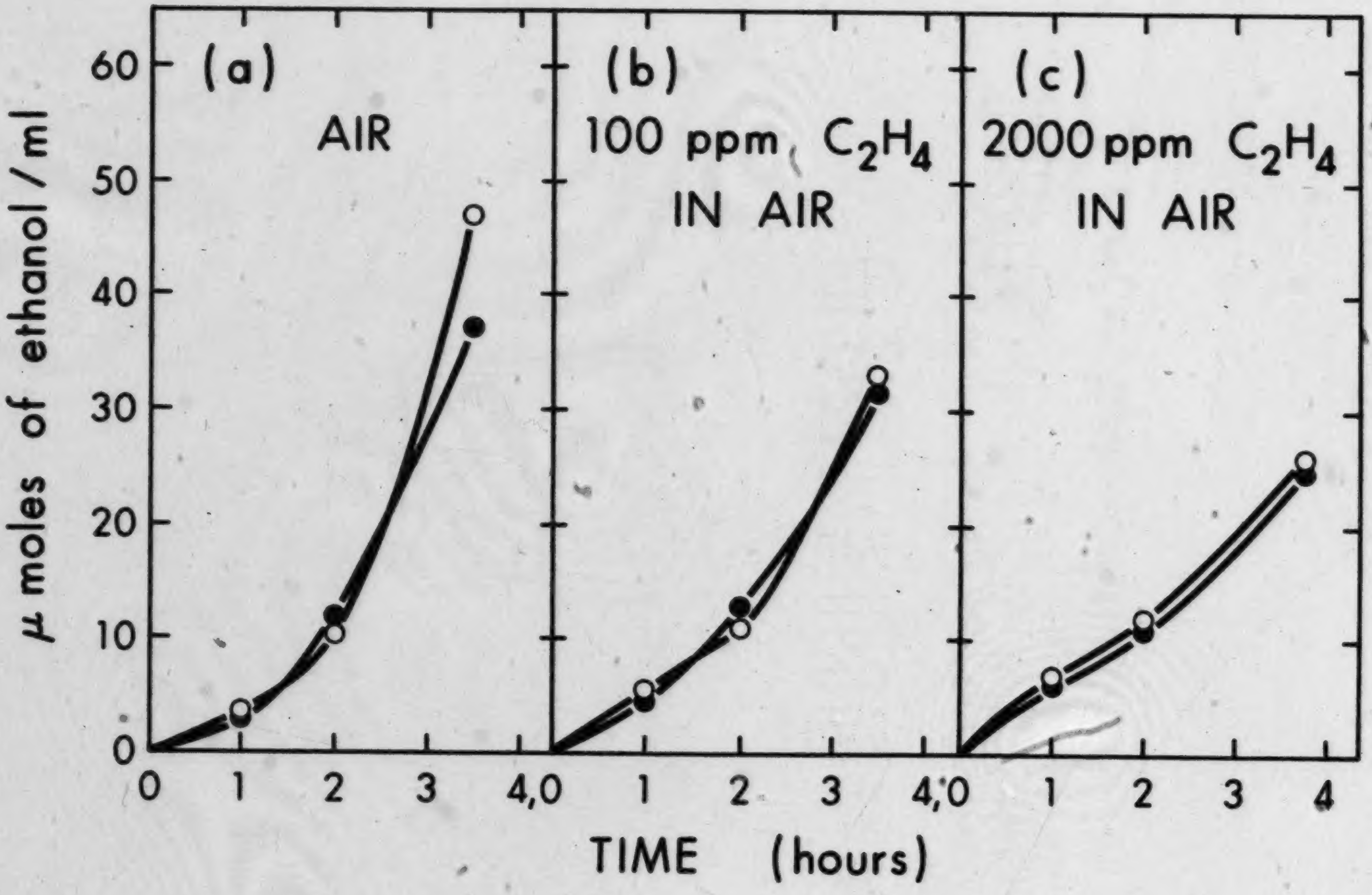
Metabolic CO_2 did not have a significant effect on ethanol production in ethylene-treated samples. But the ethanol production in controls was affected by metabolic CO_2. The total amount of ethanol produced was 47 μmoles/ml in the absence of metabolic CO_2, and 36 μmoles/ml in its presence. It is known that if CO_2 is allowed to build up, ethanol production by yeast will decrease (81). The concentration of CO_2 built up in ethylene treated samples was probably not sufficient to decrease ethanol production.

The effect of ethylene on fermentation of glucose by the yeast is made clear by comparing the rates of production of ethanol in samples treated with ethylene and untreated samples. Data presented in Table 10

Fig. 26. Rate of ethanol production from glucose by Saccharomyces cerevisiae (X-2180-1B) in presence and absence of applied ethylene, and the effect of metabolic CO_2 on ethanol production.

● CO_2 removed

○ CO_2 not removed


(a)
AIR
(b)
100 ppm C_2H_4
IN AIR
(c)
2000 ppm C_2H_4
IN AIR
µ moles of ethanol / ml
0
10
20
30
40
50
60
0
1
2
3
4,0
1
2
3
4,0
1
2
3
4
TIME (hours)

indicate that the rate of production of ethanol increased considerably with time when the sample was not treated with ethylene. With ethylene-treated samples the increase in the rate of ethanol production with incubation time was not to the same extent and in fact it was less at 2000 ppm C_2H_4 than at 100 ppm.

The difference in the increase in the rate of production of ethanol between controls and ethylene-treated samples is made clear by comparing the ratios of final rate (120-210 minutes) to initial rate (0-60 minutes) (Table 12). When no ethylene was added and the metabolic CO_2 was absorbed by KOH, the final rate of ethanol production increased to 683% of the initial rate. When CO_2 was not removed this increase was only 464% of the initial rate. With an ethylene concentration of 2000 ppm the final rate of ethanol production increased only to 161% of the initial rate in the presence of metabolic CO_2 and 167% in its absence. An intermediate concentration level of ethylene (100 ppm) had an intermediate effect; the final rates being 272% of the initial rate when metabolic CO_2 was trapped by KOH and 265% when CO_2 was not trapped. It should be noted, nowever, that the initial rates of production of ethanol (0-60 minutes) in ethylene-treated samples were about 50% higher than the corresponding values of the control. But the final rates in the ethylene-treated samples were much lower than the respective values for controls. These aspects are better illustrated in Figure 27.

There was a stimulation of production of ethanol during the early stages of incubation in ethylene-treated samples as compared to controls (no ethylene treatment). The initial stimulation of production of ethanol was slightly greater at an ethylene concentration of 2000 ppm

TABLE 12: Effects of Metabolic CO_2 and Exogenously Applied Ethylene on Rate of Ethanol Production from Glucose by *Saccharomyces cerevisiae* (X-2180-1B)

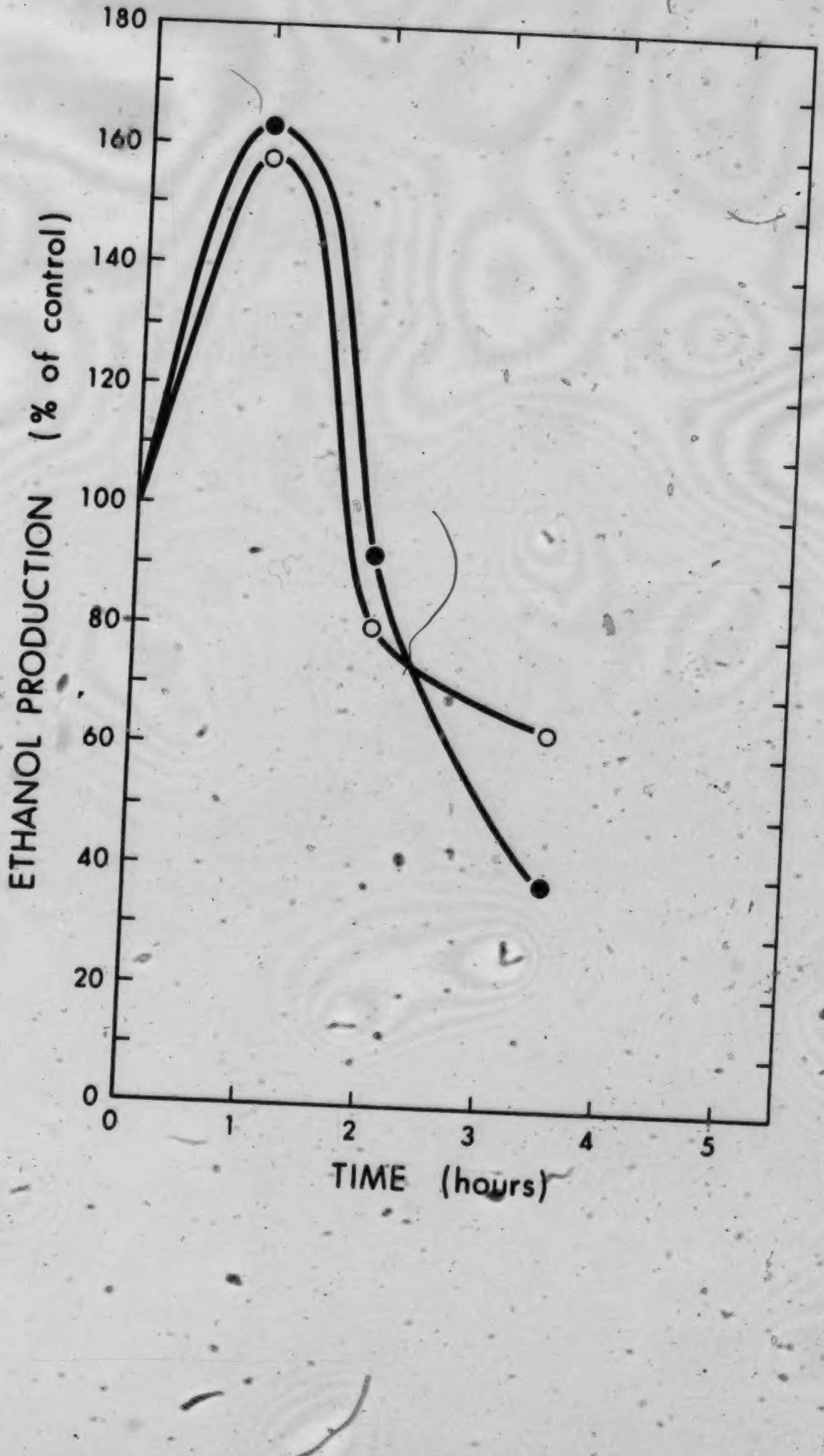
Concentration of Ethylene in Air (ppm)	Metabolic CO_2	(μ moles ethanol/min/ml)10^2		Ratio
		0-60 min (Initial)	120-210 min (Final)	$\left(\frac{\text{Final Rate}}{\text{Initial Rate}}\right)$
0	Removed	5.9	40.4	6.83
	Not Removed	5.8	27.1	4.64
100	Removed	9.3	25.4	2.72
	Not Removed	8.4	22.4	2.67
2000	Removed	9.6	15.4	1.61
	Not Removed	10.2	16.9	1.67

Mid-log phase cells obtained by growing the yeast in lactate medium were used in this experiment. The cells were suspended in 0.1 M potassium phosphate buffer (pH 5.0) containing 5% glucose. The suspension was distributed in 20 ml quantities. The flasks were flushed with appropriate concentrations of ethylene for 5 min, and then sealed. Metabolic CO_2 was absorbed by KOH placed in a Scintillation vial inside the flask. Ethanol content at various time intervals determined enzymatically as described in Materials and Methods.

Fig. 27. Rate of ethanol production from glucose by *Saccharomyces cerevisiae* (X-2180-1B) in presence and absence of applied ethylene. The results expressed as % of control (no ethylene treatment).

● 2000 ppm ethylene in air

○ 100 ppm ethylene in air


ETHANOL PRODUCTION (% of control)
TIME (hours)
180
160
140
120
100
80
60
40
20
0
0
1
2
3
4
5

than at 100 ppm. Also at the higher concentration of ethylene (2000 ppm) the increase in the rate of production of ethanol on prolonged incubation was inhibited to a greater extent than with the lower concentration of ethylene (100 ppm).

5. Effect of Ethylene on $^{14}CO_2$ Production by Yeast from Glucose-3,4-^{14}C

The fact that treatment of the yeast with ethylene affects its capacity to produce ethanol from glucose was further investigated by determination of the rate of $^{14}CO_2$ production from specifically labelled radioactive glucose. Fermentation of glucose to ethanol results in the conversion of the third and fourth carbon atoms of glucose to carbon dioxide. Therefore, the rate of production of ethanol from glucose should be parallel to the rate of production of CO_2 from third and fourth carbon atoms of glucose. This was studied by determining the rate of production of $^{14}CO_2$ from glucose-3,4-^{14}C by the yeast and comparison of results with those obtained for ethanol production. The radiorespirometry used in the determination of $^{14}CO_2$ is described in Materials and Methods. Results are presented in Figure 28 and Table 13.

The initial rate (0-30 minutes) of production of $^{14}CO_2$ from glucose-3,4-^{14}C by the yeast was 90% higher in the presence of 100 ppm ethylene in air, than in its absence (Figure 28). On further incubation, however, the stimulatory effect of ethylene on $^{14}CO_2$ production diminished and eventually ethylene seemed to inhibit $^{14}CO_2$ production by the yeast.

The data presented in Table 13 indicate that the net uptake of glucose-3,4-^{14}C by the yeast was less in the presence of ethylene than in its absence. With ethylene treatment nearly 25% of the added radio-

Fig. 28. $^{14}CO_2$ prodution by Saccharomyces cerevisiae (X-2180-1B) from glucose-3,4-^{14}C, in presence and absence of 100 ppm ethylene in air. Washed yeast cells (86 mg dry wt) were suspended in 15 ml of 0.1 M potassium phosphate buffer (pH 5.0) in a radiorespirometric flask. To each flask 4.2 mmoles of glucose-3,4-^{14}C (specific activity 104,760 dpm/mmole) were added. The $^{14}CO_2$ produced was determined as described in Materials and Methods. The results are expressed as percetage of control (no ethylene treatment).

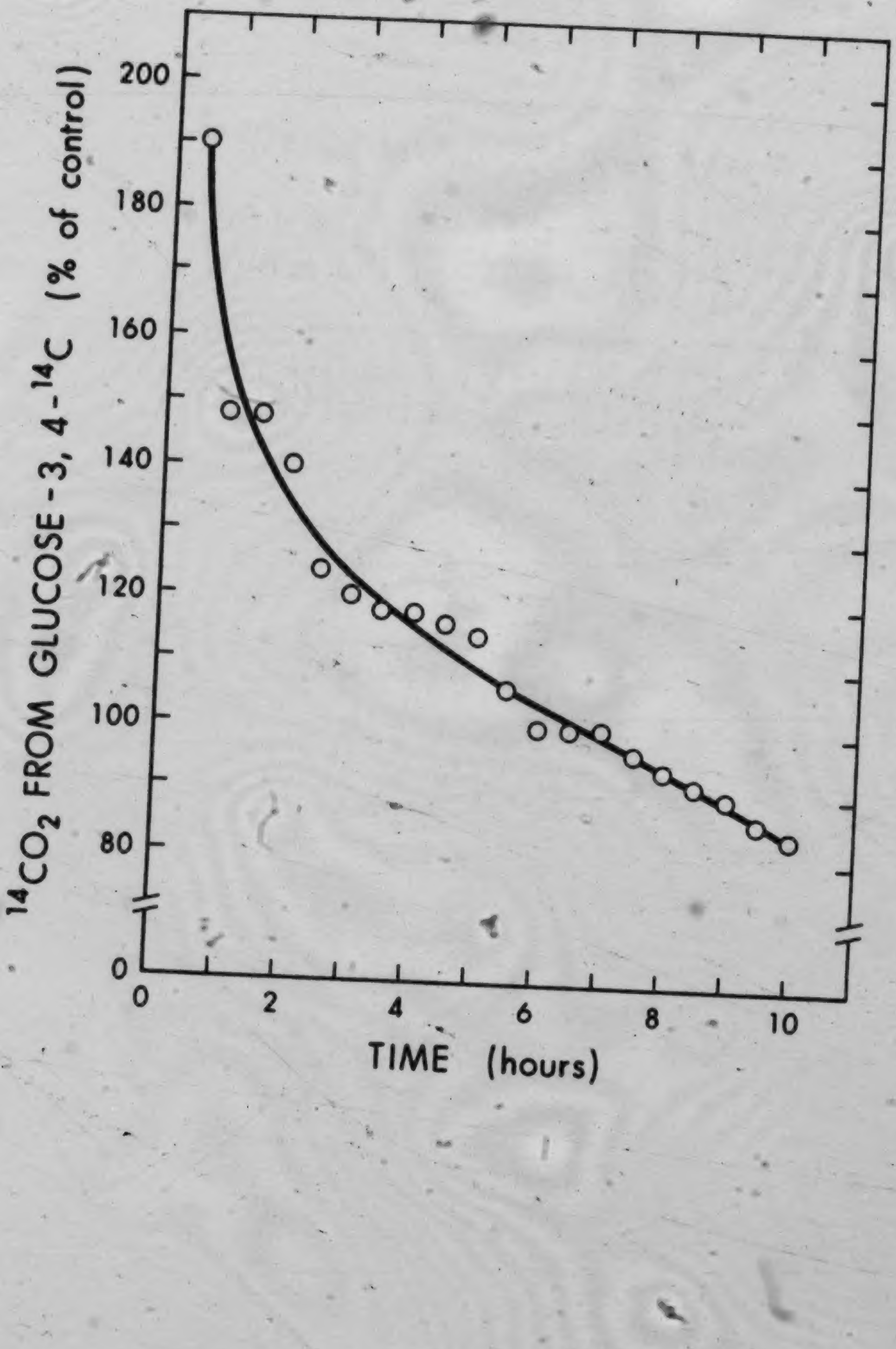

$^{14}CO_2$ FROM GLUCOSE-3, 4-^{14}C (% of control)
200
180
160
140
120
100
80
0
0
2
4
6
8
10
TIME (hours)

TABLE 13: Effect of 100 ppm Ethylene in Air on Uptake and Metabolism of Glucose-3,4-^{14}C by Saccharomyces cerevisiae (X-2180-1B).

Treatment	Total dpm added as Glucose-3,4-^{14}C	dpm Recovered				% Recovery
		in Cells	in Medium	as $^{14}CO_2$	Total	
Air (Control)	404,340	14,280	50,600	341,305	406,185	100.5
100 ppm Ethylene in	404,340	6,480	110,800	278,565	395,485	97.9
% of Control	-	45	219	82	-	-

Log-phase cells obtained by growing the yeast in lactate medium were suspended in 0.1 M potassium phosphate buffer (pH 5.0). The $^{14}CO_2$ production by the yeast from glucose-3,4-^{14}C determined by radiorespirometry as described by Wang (193). The reaction flask contained 15 ml cell suspension (86 mg dry wt) and 4.2 m moles of glucose-3,4-^{14}C (Sp. activity 104,760 dpm/m mole). Total duration of the experiment was 10 hours.

activity was left in the medium at the end of 10 hours, whereas in the absence of applied ethylene the corresponding value was 13%. Also the incorporation of radioactivity into the yeast cells was decreased by 55% with ethylene treatment. These data clearly indicate the exogenously applied ethylene affects the uptake of glucose by the yeast. The effect of ethylene on glucose uptake by the yeast was studied by direct methods described in the next section.

6. Effect of Ethylene on Uptake of Glucose by Saccharomyces cerevisiae (X-2180-1B)

Results presented in Figure 29 indicate that treating the yeast with ethylene resulted in a fast uptake of glucose initially. But on continued incubation the amount of glucose taken up by the yeast in the presence of applied ethylene did not increase as rapidly as in the absence of applied ethylene. The amount of glucose taken up during 60 minutes by the yeast in the presence of 100 ppm ethylene in air was 47% less than that taken up by the yeast in the absence of applied ethylene.

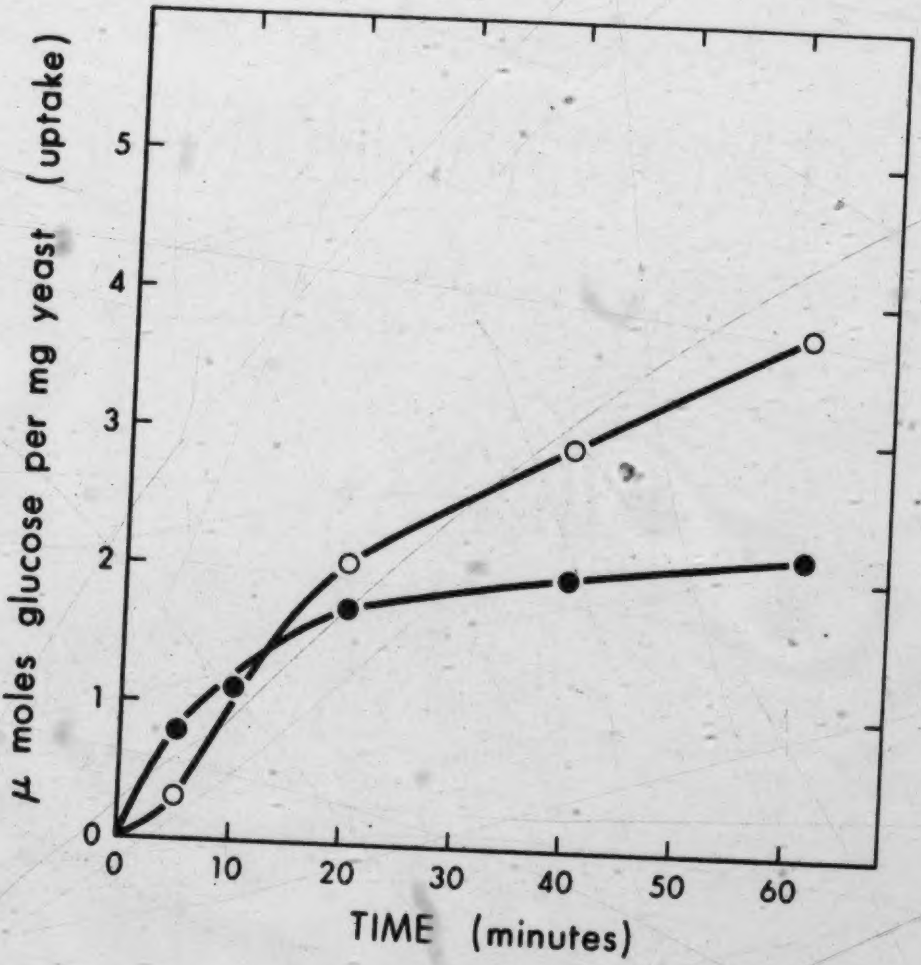
Ethylene seems to have similar effects of glucose uptake (Figure 29) and ethanol production (Figure 26) by *S. cerevisiae*. Thus the decreased production of ethanol by the ethylene-treated yeast may be attributable to decreased uptake of glucose. The lack of a Crabtree effect at higher concentrations of glucose (Figure 23) also might result from decreased uptake of glucose by the ethylene-treated yeast.

The rapid utilization of D-glucose by yeast raises the possibility that the rate of disappearance of glucose from the suspending medium is a reflection of the rate at which it is metabolized rather than the transport process *per se*. The latter possibility can be tested

Fig. 29. Effect of ethylene on uptake of glucose by Saccharomyces cerevisiae (X-2180-1B). Mid-log phase cells obtained by growing the yeast in lactate medium were suspended in 0.1 M potassium phophate buffer pH 5.0 and glucose was added to the suspension to give a concentration of 0.5% . The suspension was aerated with air or 100 ppm ethylene in air at the rate of 50 ml/min. At various intervals glucose remaining in the medium was determined enzymatically.

● 100 ppm ethylene in air

○ Air


μ moles glucose per mg yeast (uptake)
0
1
2
3
4
5
0
10
20
30
40
50
60
TIME (minutes)

by investigating the uptake of non-metabolizable analogue of glucose, 3-O-methyl glucose. Kinetics of 3-O-methyl glucose uptake by S. cerevisiae (X-2180-1B) were studied, under the same experimental conditions as in the sutdy of glucose uptake.

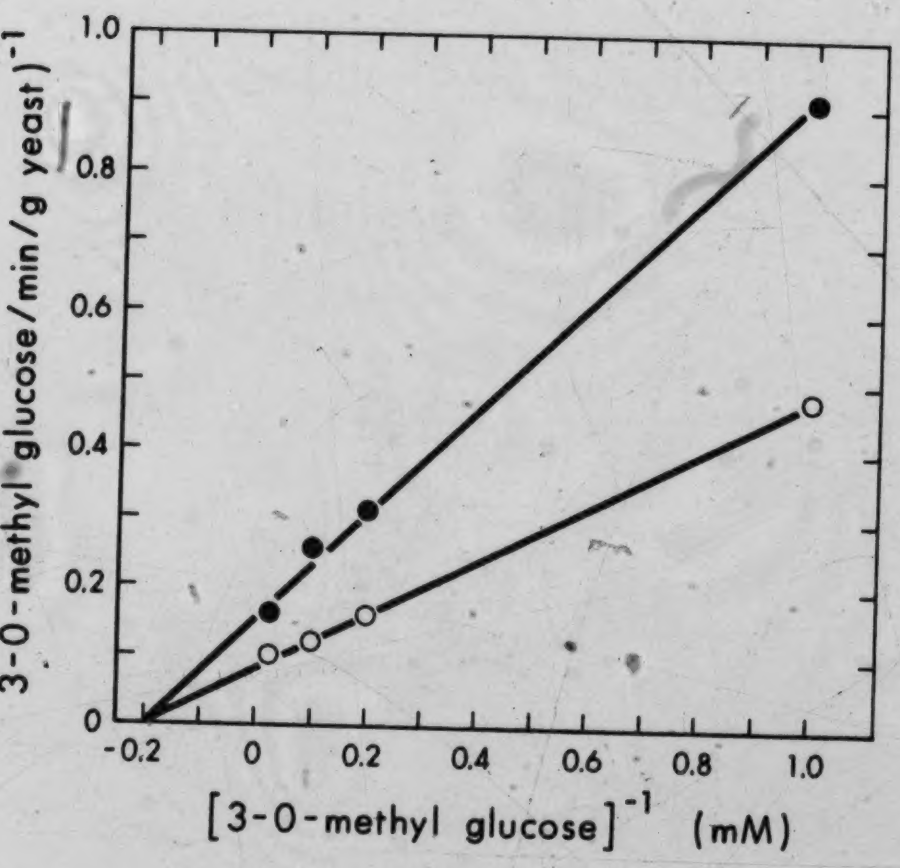
A Lineweaver-Burk plot of the data (Figure 30) indicates that the Km for the uptake of 3-O-methyl glucose by the yeast was not affected by the ethylene treatment. The Km was found to be 5 mM 3-O-methyl glucose, with and without ethylene treatment. But the Vmax for 3-O-methyl glucose transport was decreased by treatment of the yeast with ethylene. Unaffected Km and decreased Vmax are features of non-competitive inhibition (180). Therefore the decreased uptake of 3-O-methyl glucose by the yeast may be a result of non-competitive inhibition of the transport system by ethylene. That ethylene inhibits uptake of glucose by the yeast after a certain length of time of ethylene application (previous two sections) is further supported by these findings. Since 3-O-methyl glucose is not metabolized by the yeast, the inhibition by ethylene of 3-O-methyl glucose (and most likely D-glucose as well) transport is independent of its metabolic rate.

It was reported (Figure 23) that the respiration of the yeast in the presence of glucose was stimulated by exogenously applied ethylene. Whether the decreased uptake of glucose by the yeast in presence of ethylene is a result of increased respiration is not known. In this respect it is interesting to note that in yeast the affinity of the uptake system for glucose is lower under aerobic than it is under anerobic conditions (76). A similar observation has been made on sugar transport in avian erythrocytes by Whitfield and Morgan (196).

Fig. 30. Double reciprocal plot of 3-O-methylglucose uptake by *Saccharomyces cerevisiae*(X-2180-1B) in presence and absence of 100 ppm ethylene in air.

● 100 ppm ethylene in air

○ Air


1.0
0.8
0.6
0.4
0.2
0
-0.2
0
0.2
0.4
0.6
0.8
1.0
(μ moles 3-0-methyl glucose/min/g yeast)$^{-1}$
[3-0-methyl glucose]$^{-1}$ (mM)

7. Effect of Ethylene on the Synthesis of Glycolylic Enzymes

Ethylene is reported to preferentially stimulate Embden-Meyerhof-Parnas (EMP) pathway in ripening banana (175) and in carrots (154) by shifting the balance between the EMP pathway and pentose phosphase pathway in favor of the former. Hartman (63) suggested that pentose cycle might operate in preclimacteric fruits (banana, apple and pear) and then with the onset of ripening would decrease with a shift towards glycolysis. Whether ethylene stimulates glucolysis through increased synthesis of glycolytic enzymes or through modulation of the activities of regulatory enzymes is not known. Therefore, an attempt was made to measure the amounts of some glycolytic enzymes in yeast grown in the presence, or in the absence, of ethylene.

Saccharomyces cerevisiae (X-2180-1B) was grown in lactate medium to the mid-log phase with air or 100 ppm ethylene in air bubling through the growth medium at the rate of 100 ml per minute. Enzymes from the cells were extracted by treating them with toluene, and their activities determined fluorometrically by coupling reactions as described in Materials and Methods. Since the enzymes were assayed under identical conditions, a difference in specific activities between yeast grown in the presence of air and 100 ppm ethylene in air was taken as the difference in the amount of enzyme present. No attempt was made to distinguish between increased specific activity and increased enzyme synthesis.

Results presented in Table 14 indicate that in the extract of yeast grown in the presence of ethylene all enzymes tested showed greater activity per mg protein of extract than when the yeast was grown in the absence of ethylene. The hexokinase (EC 2.7.1.1) content in the yeast

TABLE 14: Effect of Growing Saccharomyces cerevisiae (X-2180-1B)in the Presence of 100 ppm Ethylene in Air on Synthesis of Certain Enzymes

Enzyme	munits of enzyme/mg protein		% of control
	Air(control)	100 ppm ethylene in air	
Hexokinase	1194	1421	119
Phosphofructokinase	94	151	160
Glyceraldehyde-3-P dehydrogenase	194	301	155
Pyruvate kinase	7632	9946	130
Alcohol dehdrogenase	4163	5611	134
Glucose-6-P dehydrogenase	305	373	122
6-Phosphogluconate dehydrogenase	264	337	127

The yeast was grown in lactate medium in the presence of air or 100 ppm ethylene in air. Enzymes from the cells were extracted by toluene treatment. Activities of enzymes were determined as described in Materials and Methods. munit = nmoles substrate converted per minute.

seemed to be affected by ethylene only to a small extent (19% increase). Phosphofructokinase (EC 2.7·1·11), on the other hand, was increased by 60% in the yeast grown in the presence of 100 ppm ethylene. Contrary to the expectation, the specific activities of the pentose cycle enzymes [Glucose-6-phosphate dehydrogenase (EC 1·1·1·49) and gluconate-6-phosphate dehydrogenase (EC 1.1.1.44)] in the yeast were also stimulated by ethylene. Therefore, growing the yeast in the presence of ethylene seems to increase synthesis of glycolytic as well as pentose cycle enzymes. This suggests that the yeast grown in the presence of ethylene has a greater capacity to dissimilate glucose through EMP and pentose phosphate pathways than the yeast grown in its absence. But the activity of the pentose cycle has been shown to decrease in the presence of applied ethylene in banana and carrots (154, 175) and in ripening fruits which produce ethylene themselves (63). The decreased flow of glucose through the pentose cycle in the presence of ethylene, suggest that controls of the cycle activity by means other than through regulation of enzyme synthesis, may exist. In apples, application of ethylene has been shown to stimulate decarboxylation of malate through increased synthesis of malic enzymes (147). The reaction catalyzed by this enzyme also provides NADPH, needed in several biosynthetic reactions. Therefore, in the presence of applied ethylene much of the NADPH is probably derived from the reaction catalyzed by malic enzyme and not from the pentose phosphate pathway. Also the decreased activity of the pentose phosphate pathway in the presence of ethylene may result from the inhibitory action of NADPH produced by malic enzyme, since it has been shown that NADPH inhibits 6-phosphogluconate dehydrogenase (104), the regulatory enzyme in the pentose phospate pathway.

8. Effect of Ethylene and Glucose on Intracellular Levels of Glucose-6-Phosphate and Adenosine Triphosphate

It was shown in the previous section that ethylene stimulated the synthesis of a number of glycolytic enzymes in yeasts grown in lactate medium. Glucose also induces synthesis of glycolytic enzymes in yeast (102) and the actual inducer is thought to be the product or a derivative of the product of hexokinase reaction (104). The rate of glycolysis in yeast is dependent on the degree of induction of synthesis of glycolytic enzymes and the availability of ATP for glucose phosphorylation. In the following set of experiments, the effects of ethylene and glucose on intracellular levels of glucose-6-phosphate (G6P) (the probable inducer of glycolysis) and ATP in yeast were studied. The data presented in Figures 31 and 32 are average values of determinations made in triplicate. The results were normalized for differences in cell mass per ml of culture by dividing the data by the respective absorbances at 650 nm.

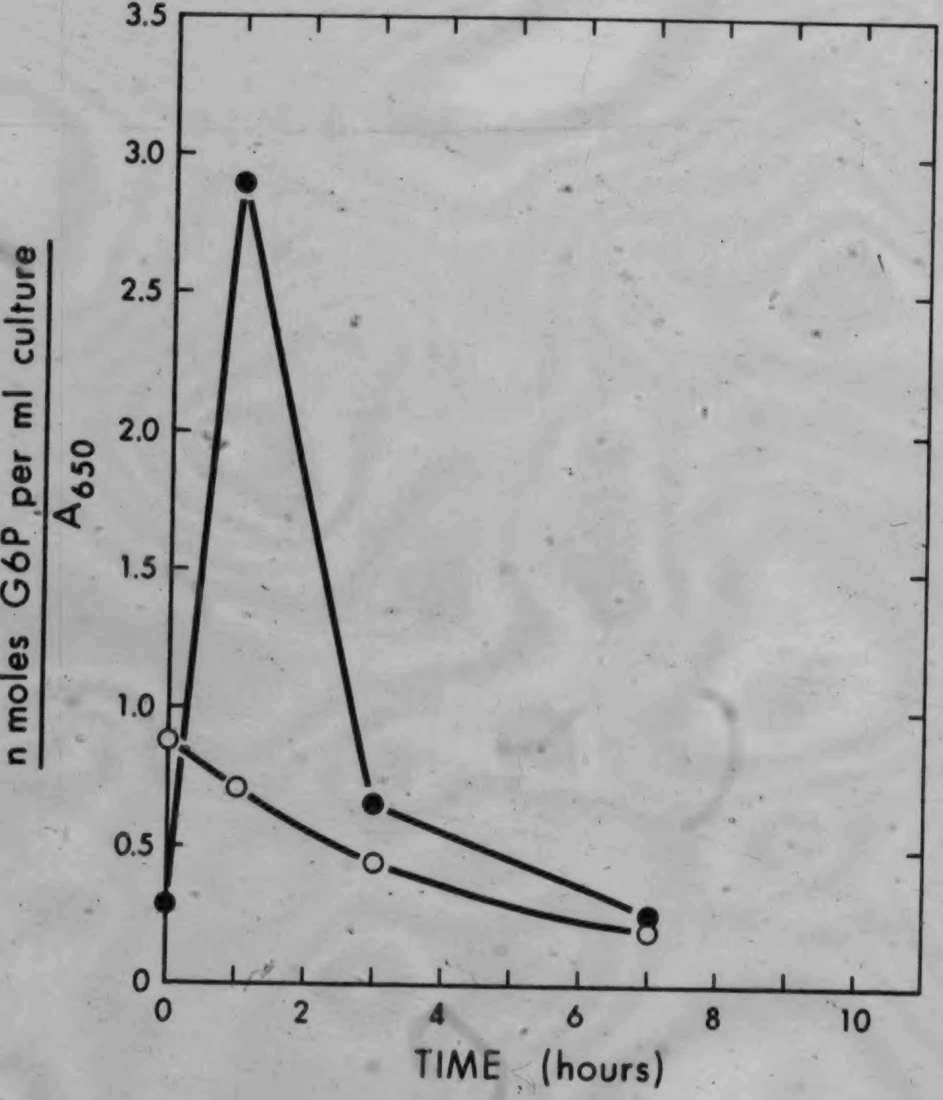
The initial G6P content of the yeast grown in the presence of ethylene was 67% less than that found in the yeast grown in the absence of ethylene (Figure 31). But on addition of glucose, the level of G6P in ethylene-treated yeast increased greatly during the early stages of incubation. The G6P content of the ethylene-treated yeast, 60 minutes after the addition of glucose was four times as much as found in non-ethylene-treated yeast. This initial increase in the G6P content in the ethylene-treated yeast corresponds to the initial increase in the glucose uptake (Figure 29) and ethanol production (Figure 25) by the yeast in presence of applied ethylene.

On subsequent incubation, the amount of G6P per unit weight of yeast decreased, and seven hours after the addition of glucose, there

Fig. 31. Effect of glucose and 100 ppm ethylene in air on intracellular levels of glucose-6-phosphate in Saccharomyces cerevisiae (X-2180-1B). The yeast was grown in lactate medium to the mid-log phase in presence or absence of 100 ppm ethylene in air. Glucose (5%) was added at zero time and the G-6-P concentrations in yeast were determined as described in Materials and Methods.

○ Treated with air

● Treated with 100 ppm ethylene in air


3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
0
2
4
6
8
10
TIME (hours)
n moles G6P per ml culture / A_{650}

was practically no difference between G6P contents of yeasts grown in presence and absence of ethylene. This is in agreement with the results presented in Figure 24 where it was shown that ethylene did not have any apparent effect on ethanol production or glucose uptake by the yeast growing in glucose medium. Ethylene seems to affect yeast metabolism only during the early stages of adaptation to glucose.

The ATP level of the yeast before the addition of glucose was less with ethylene-treatment than without (Figure 32). This is in agreement with the results presented in Table 11. On addition of glucose, the ATP levels in ethylene-treated yeast decreased after an initial rise. In the absence of ethylene the trend was the same, except that there was no initial rise in the levels of ATP in the cells. The fast decrease of ATP in the cells on addition of glucose may result from it being used for the phosphorylation of glucose.

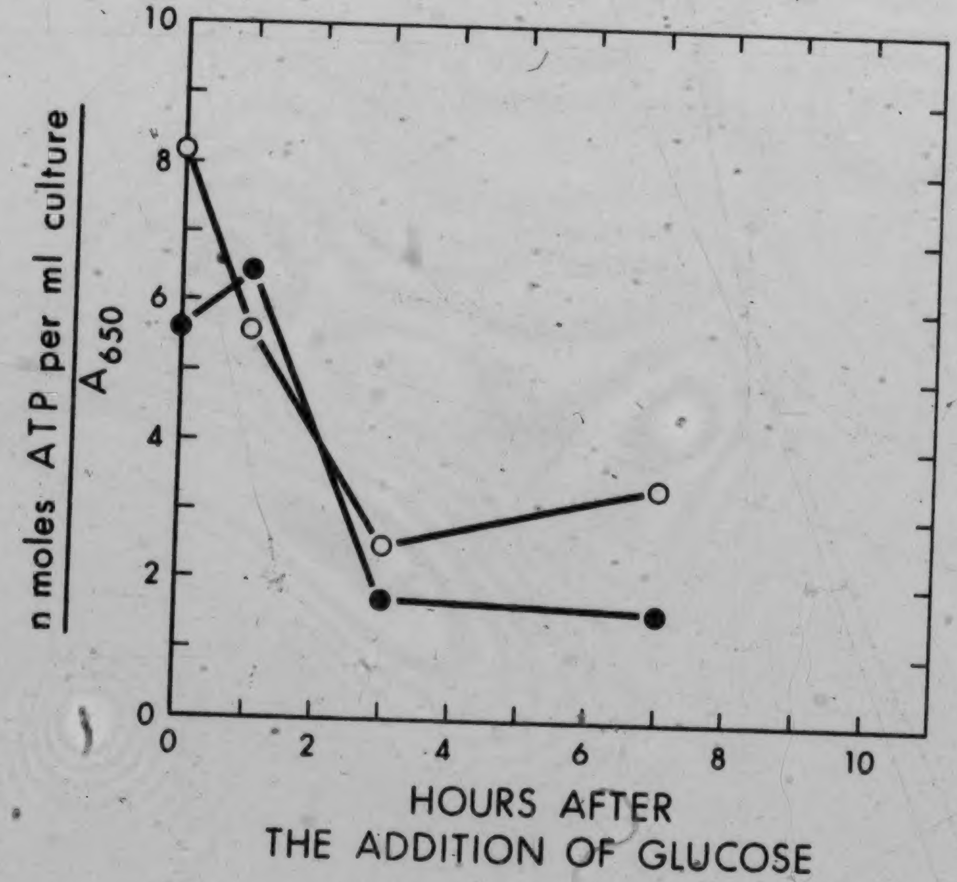
9. Effect of Ethylene and Carbon Dioxide on K^+ Transport in Saccharomyces cerevisiae (X-2180-1B)

The influx of K^+ is an active transport process in yeast. Commercial baker's yeast takes up K^+ from the medium even against a concentration gradient in excess of 1000:1 (174). Uptake of potassium ions is connected with the transport of H^+ from the cell, which may occur against a gradient of 50:1 (149). Influx of K^+ is associated with the assimilation of a metabolizable substrate (174). Ryan et al. (151) reported that addition of acetate of propionate to yeast that was oxidizing ethanol in a buffer at pH 4.75, resulted in increased rate of K^+ uptake. They also observed that bubbling the suspension with CO_2 could increase the rate of influx of K^+ in the absence of any added acetate of propionate. They

Fig. 32. Effect of glucose and 100 ppm ethylene in air on intracellular levels of ATP in *Saccharomyces cerevisiae* (X-2180-1B). The yeast was grown in lactate medium to the mid-log phase in presence or absence of 100 ppm ethylene in air. Glucose (5%) was added and the ATP concentrations in yeast were determined as described in Materials and Methods.

○ Treated with air

● Treated with 100 ppm ethylene in air


n moles ATP per ml culture / A$_{650}$
10
8
6
4
2
0
0
2
4
6
8
10
HOURS AFTER
THE ADDITION OF GLUCOSE

concluded from these results that the intracellular pH was the major factor in the control of K^+-H^+ exchange system.

Ryan and Ryan (150) found that yeast did not take up K^+ when the substrate was propan-2-ol (isopropanol). However, when the suspension was bubbled with a mixture of CO_2 and O_2 considerable amounts of K^+ were taken up. The intracellular pH of the yeast is in equilibrium with the pH of the suspending medium. A decrease in the pH of the suspending medium brought about by CO_2 is thought to be responsible for the influx of K^+.

Yeast produces large amounts of CO_2 under suitable growth conditions and CO_2 in the atmosphere had been shown to affect yeast metabolism (81).

In the following set of experiments the effect of ethylene on CO_2-stimulated K^+ uptake by S. cerevisiae (X-2180-1B) was studied. The yeast treated with CO_2-free air or 0.2% (2000 ppm) ethylene in CO_2-free air did not take up K^+. The data presented in Figure 33 are typical of results obtained in several experiments.

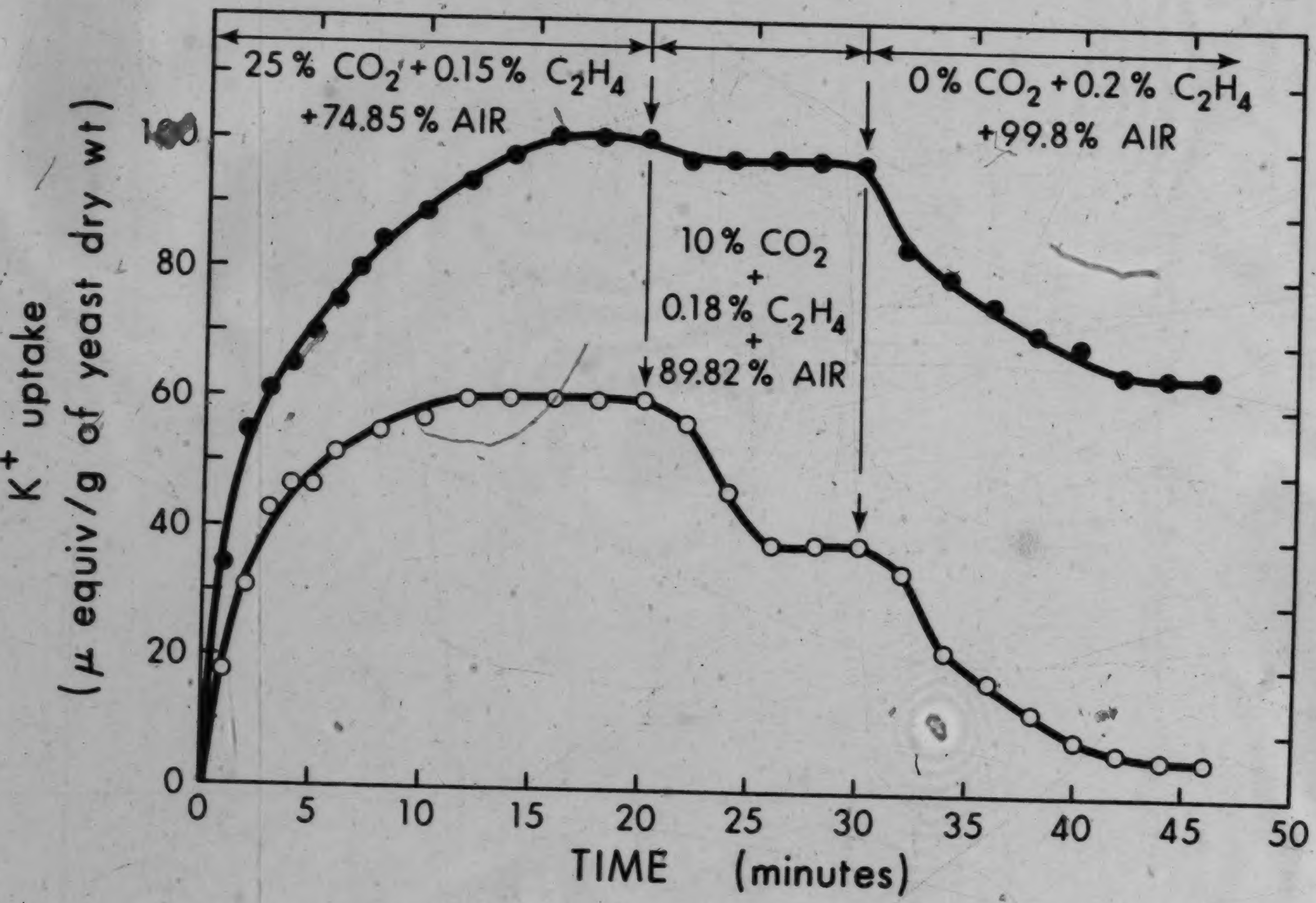
Bubbling the cell suspension with a gas mixture (100 ml per min) containing 25% CO_2 resulted in rapid uptake of K^+ by the yeast. This uptake was further stimulated by 0.15% (1500 ppm) ethylene in the gas mixture. The total amount of K^+ taken up by the yeast reached a steady value by 15 minutes. There was a large efflux of K^+ from the yeast on reducing concentration of CO_2 in the gas mixture from 25% to 10%. This efflux was considerably smaller when the gas mixture contained ethylene. When ethylene was not present in the atmosphere removing CO_2 completely from the gas mixture resulted in efflux of all of K^+ that was

Fig. 33. Effect of carbon dioxide and ethylene on K^+ uptake by *Saccharomyces cerevisiae* (X-2180-1B). The yeast cells were suspended in 0.02 M citrate buffer (pH 4.75) containing 0.5 mM KCl. Uptake of K^+ was initiated by bubbling with various concentrations of carbon dioxide or carbon dioxide plus ethylene.

○ Carbon dioxide + air

● Carbon dioxide + ethylene + air

↓ Point at which the concentration of carbon dioxide was changed


25 % CO_2 + 0.15 % C_2H_4
+74.85 % AIR
10 % CO_2
+
0.18 % C_2H_4
+
89.82 % AIR
0 % CO_2 + 0.2 % C_2H_4
+99.8 % AIR
K^+ uptake
(μ equiv/g of yeast dry wt)
0
20
40
60
80
100
0
5
10
15
20
25
30
35
40
45
50
TIME (minutes)

taken up. But in the presence of ethylene only a portion of K^+ taken up was lost from the yeast by the removal of CO_2 from the gas mixture.

The increased uptake and retention of K^+ by the yeast treated with a mixture of CO_2, ethylene and air may reflect its metabolic state. According to Ryan and Ryan (150) it is the intracellular pH that determines the rate of influx of K^+. If this is so, one can expect a greater change in the intracellular pH of the yeast treated with ethylene in presence of CO_2 and air, than the yeast treated with carbon dioxide and air alone. Measurement of the intracellular pH under the above experimental conditions was found to be difficult and therefore was not attempted.

V GENERAL DISCUSSION AND CONCLUSIONS

Results reported in this thesis clearly indicate that the production of ethylene by Saccharomyces cerevisiae was dependent on the composition of the growth medium. There was no net production of ethylene when lactate was the major carbon source in the medium. Under these conditions the yeast seemed to absorb ethylene from the surrounding medium (Table 1).

Whether ethylene is a required metabolite for the yeast could not be studied, since it was impossible to devise a growth medium that did not itself produce trace amounts of ethylene. Nevertheless, it is interesting to note that ambient levels of ethylene play a role in the development of plants. Abeles et al. (8) reported that the synthesis of β-1,3-glucanase in bean leaves was influenced by ambient levels of ethylene and removal of ethylene greatly decreased the β-1,3-glucanase content. Shimoda and Yanagishima (157) found that the application of exo-β-glucanase to S. cerevisiae caused cell expansion. Interestingly this response to glucanase was directly related to the ability of yeast strains to elongate under the influence of auxin (indoleacetic acid). These results tend to parallel findings made on cells of higher plants where indoleacetic acid has been found to induce β-glucanase (45). Since exogenous application of β-1,3-glucanase caused the cell elongation of oat coleoptile as well as cell expansion in S. cerevisiae, it was proposed that a common mechanism may exist for the auxin-induced elongation of yeasts and higher plants (157). The common mechanism may be mediated through the action of ethylene, since auxin is known to stimulate ethylene production (187) and ethylene is reported to induce synthesis of β-glucanases (8). Thus it is interesting to speculate that ethylene absorbed from the medium may play a role in the induction of β-1,3-glucanase in yeast.

Addition of glucose to the yeast growing in lactate medium induced ethylene production and this induction probably involved de novo synthesis of enzyme or enzymes since cycloheximide almost completely inhibited glucose-induced synthesis of ethylene (Figure 1, 2 and Table 2). Whether the stimulatory effect of glucose on ethylene production through generation of H_2O_2 could not be answered unequivocally. Direct addition of H_2O_2 had a transient stimulatory effect on ethylene production (Table 6) Addition of glucose + glucose oxidase resulted in a considerable stimulation of ethylene production (Figure 16 and Table 6). This stimulatory effect did not appear to be a result of H_2O_2 since addition of catalase did not prevent the increased ethylene production caused by glucose + glucose oxidase.

One noticeable result of addition of glucose to the yeast growing in lactate medium was an immediate decrease in the intracellular concentration of L-alanine (Table 7). Exogenously applied L-alanine inhibited glucose-induced ethylene production. One reason, therefore, for the lack of ethylene production by the yeast growing in lactate medium may be the high intracellular content of L-alanine. Whether L-alanine could inhibit ethylene production by the yeast when ethylene synthesis occurs at maximal rate in the presence of added glucose and L-methionine is worth investigating.

The reason for the decreased ethylene production with increasing glucose concentration (Figure 3) is not clearly understood. It may be related to the rates of growth of the organism in different concentrations of glucose. . Lynch and Harper (94) recently showed that the rate of ethylene production by Mucor hiemalis was inversely proportional to the rate of growth of the organism. Whether glucose in the presence of lactate became a limiting nutrient was not tested.

While ethylene production was stimulated by oxygen, significant amounts of ethylene were produced even in the absence of oxygen (Figure 4). Since oxygen stimulated production of ethylene in the yeast filtrate it appeared that a precursor of ethylene was synthesised by the yeast and excreted into the medium. The conversion of this precursor to ethylene was stimulated by oxygen. Accumulation of an ethylene precursor in the absence of oxygen has been observed in fruits (15, 35). But unlike fruits, the yeast continued to synthesise ethylene under anaerobic conditions although the amount of ethylene thus produced was less than that produced in aerobic conditions.

Since respiration deficient mutants of S. cerevisiae produced ethylene (Table 3), the suggestion (118, 119, 120) that respiration and ATP generated through mitochondrial oxidative phosphorylation are closely linked to the biosynthetic pathway of ethylene does not seem to apply in the case of yeast.

Ethylene production by S cerevisiae growing in lactate medium was stimulated not only by glucose but also by L-methionine (Figure 6). When the growth medium contained both glucose and L-methionine, the rates of ethylene production were considerably higher than when either compound was omitted from the growth medium (Figures 7, 8 and Table 5). This observation is in agreement with the findings of Lynch (95) who reported that the rate of production of ethylene by Mucor hiemalis was maximal only when the growth medium contained both glucose and L-methionine. Increasing the L-methionine concentration from 1 mM to 5 mM increased the rate of ethylene production even further (Figure 10).

That ethylene was produced by an adenine- and methionine-requiring auxotroph of _S. cerevisiae_ in the presence of L-methionine but not in its absence (Fig. 19) indirectly suggested L-methionine might be a required precursor of ethylene in the yeast. Radioactive tracer studies (Table 8) with L-methionine-U-^{14}C confirmed this. The incorporation of radioactivity from L-methionine-U-^{14}C was 48% within 3 hours of incubation. This high rate of conversion of L-methionine to ethylene is in direct contrast to the low conversion observed in _Penicillium digitatum_ (2). In _Penicillium_ the immediate precursor of ethylene is thought to be isocitrate (71) or glutamic acid (40) and not L-methionine. Therefore, as in higher plants (26), a precursor of ethylene in _S. cerevisiae_ seems to be L-methionine and the biosynthetic pathway in this organism distinctly differs from that of _P. digitatum_.

The pathway of conversion of L-methionine to ethylene is not clearly understood. The possibility that L-methionine is first converted to S-adenosylmethionine which through some unknown reaction mechanism is converted to ethylene (26) has to be discounted because L-ethionine, a competitive inhibitor of methionine adenosyl transferase (41) also stimulated ethylene production by the yeast (Fig. 12). The stimulation of ethylene production by D-methionine also argues against the participation of S-adenosylmethionine as an intermediate in the conversion of L-methionine to ethylene.

The stimulatory effect of D-methionine and L-ethionine on ethylene production by the yeast may result from their being converted to ethylene as observed in plant tissues (108).

The conversion of L-methionine, D-methionine and L-ethionine to ethylene may occur through a common mechanism. In this respect, N-acyl derivatives of methionine or ethionine as possible precursors of ethylene in living organisms, are worth considering. The initial rate of ethylene production by *S. cerevisiae* in the presence of N-formylmethionine is 2.6 times faster than in the presence of L-methionine itself (Figure 20). This observation suggests that N-formylmethionine (or N-acyl derivatives of methionine) may be more immediate precursors of ethylene than methionine itself. Further support for such a conclusion comes from the observations that the acidic metabolite of D-methionine in plants is N-malonylmethionine (73) and in model systems N-formylmethionine and N-acetylomethionine yield ethylene at a faster rate than methionine itself.

When *Saccharomyces cerevisiae* was grown in the presence of applied ethylene, the capacity of the organism to respire was diminished (Figure 21). This is in contrast to the observation that exogenously applied ethylene stimulates respiration in plant tissues and in mature fruits (21). The decreased respiration of the yeast grown in the presence of ethylene might result from the reduced ATP and ADP contents of the cells (Table 9).

The endogenous respiration of the yeast that was starved for a short duration of time in the presence of 100 ppm ethylene in air remained higher than the respiration of the yeast starved in its absence (Figure 22). This indirectly suggested that treatment of the yeast with ethylene might have caused activation of adenosine triphosphatase, as in plant tissues (128, 129, 138) or that increased mobilization of endogenous substrates for respiration occurred with ethylene treatment.

Respiration of the yeast starved previously in presence of ethylene was a function of the glucose concentration in the medium, and a significant portion of this respiration was resistant to 10^{-4} M KCN (Figure 23). In the absence of ethylene treatment, the yeast exhibited the Crabtree effect with increasing glucose concentration, and the respiration became completely sensitive to inhibition by 10^{-4} M KCN. Confering sensitivity to inhibition by cyanide was a function of the concentration of glucose in the medium. Lack of the Crabtree effect and incomplete inhibition of respiration by cyanide suggested that uptake of glucose by the yeast was impaired by ethylene treatment.

Direct measurement of glucose uptake by yeast showed that ethylene treatment resulted in decreased assimilation of glucose from the medium (Figure 29). This was further confirmed by radiorespirometric studies using glucose-3,4-^{14}C (Figure 28 and Table 13). The rapid utilization of D-glucose by yeast raises the possibility that the rate of disappearance of glucose from the suspending medium is a reflection of the rate at which it is metabolized rather than the transport process per se. Since the rate of uptake of 3-O-methyl glucose (a non-metabolizable analogue of D-glucose) is reduced by ethylene treatment (Figure 30) it can be concluded that the rate of uptake of glucose is affected by ethylene treatment rather than its subsequent metabolism. This conclusion is further supported by the finding that the activities of enzymes of the yeast associated with glucose metabolism are not decreased by ethylene treatment (Table 14), but in fact remain at higher levels than the control. This indirectly suggests that the yeast treated with ethylene has a potential capability to metabolize glucose at a faster rate than the untreated yeast. This is confirmed by the fast initial uptake of glucose (Figures 28, 29) by the ethylene-treated yeast. The inhibition of glucose uptake by the yeast occurs only with prolonged treatment of the yeast with ethylene and it occurs through inhibition of the transport process as mentioned earlier. Treatment of pea stem sections with 10 ppm ethylene for 3 hours has been shown to inhibit the incorporation of glycerol-1-^{14}C into the phospholipid fraction and this has been attributed to ethylene affecting the permeability of the cell membrane (67). In a similar fashion the inhibition of glucose uptake by the yeast may be caused by permeability changes of membrane brought about by ethylene.

Prolonged exposure of the yeast to ethylene resulted in decreased production of ethanol from glucose (Figures 25 and 26). But the fact that the inhibition of ethanol production was preceded by an initial stimulation argues in favour of the capacity of the ethylene-treated yeast to dissimilate glucose at a fast rate compared to the control. Once again, the inhibitory effect of prolonged ethylene treatment on ethanol production from glucose may result from the decreased uptake of glucose into the cell rather than slowing of its subsequent conversion to ethanol. Therefore one of the primary effects of ethylene on yeast metabolism may be through its regulatory role on glucose uptake.

The increased uptake and retention of K^+ by the yeast treated with a mixture of CO_2, ethylene and air may reflect its metabolic state. It has been suggested (150) that it is the intracellular pH that determines the rate of influx of K^+ in yeast.

The view (126, 156) that ethylene affects the metabolism of the yeast is supported by the results presented in this thesis and is not in agreement with the assumption that ethylene does not have any effect on yeast physiology (2).

BIBLIOGRAPHY

1. Abeles, F.B. 1973. Ethylene in Plant Biology. Academic Press. New York and London. p 30-57.
2. Abeles, F.B. 1972. Biosynthesis and Mechanism of Action of Ethylene. Ann. Rev. Plant Physiol. 23:259-292.
3. Abeles, F.B. 1967. Mechanism of Action of Abscission Accelerators. Physiol. Plant. 20:442-454.
4. Abeles, F.B. 1966. Auxin Stimulation of Ethylene Evolution. Plant Physiol. 41:585-588.
5. Abeles, F.B. and B. Rubinstein. 1964. Regulation of Ethylene Evolution and Leaf Abscission by Auxin. Plant Physiol. 39: 963-969.
6. Abeles, F.B. 1967. Inhibition of Flowering in *Xanthium pensylvanicum* Walln. by Ethylene. Plant Physiol. 42:608-609.
7. Abeles, F.B. and L.E. Forrence. 1970. Temporal and Hormonal Control of β-1,3-Glucanase in *Phaseolus vulgaris* L. Plant Physiol. 45: 395-400.
8. Abeles, F.B., L.E. Forrence and G.R. Leather. 1971. Ethylene Air Pollution. Effects of Ambient Levels of Ethylene on the Glucanase Content of Bean Leaves. Plant Physiol. 48:504-505.
9. Armstrong, D.J. 1966. Hypothesis Concerning the Mechanism of Auxin Action. Proc. Nat. Acad. Sci. 56:64:66.
10. Anderson, J.W. 1968. Extraction of Enzymes and Subcellular Organelles from Plant Tissues. Phytochem. 7:1973-1988.
11. Apelbaum, A., E. Sfakiotkis and D.R. Dilley. 1974. Reduction in Extractable Deoxyribonucleic Acid Polymerase Activity in *Pisum sativum* Seedlings by Ethylene. Plant Physiol. 54:125-128.
12. Apelbaum, A. and S.P. Burg. 1972. Effect of Ethylene on Cell Division and Deoxyribonucleic Acid Synthesis in *Pisum sativum*. Plant Physiol. 50:117-124.
13. Apelbaum, A. and S.P. Burg. 1972. Effects of Ethylene and 2,4-Dichlorophenoxyacetic Acid on Cellular Expansion in *Pisum sativum*. Plant Physiol. 50:125-131.
14. Baur, A.H. and S.F. Yang. 1972. Methionine Metabolism in Apple Tissue in Relation to Ethylene Biosynthesis. Plant Physiol. 49:s-21.

15. Baur, A.H., S.F. Yang, H.K. Pratt and J.B. Biale. 1971. Ethylene Biosynthesis in Fruit Tissues. Plant Physiol. 47:696-699.

16. Baur, A.H. and S.F. Yang. 1969. Precursors of Ethylene. Plant Physiol. 44:1347-1349.

17. Bendall, D.S. and W.D. Bonner. 1971. Cyanide Insensitive Respiration of Plant Mitochondria. Plant Physiol. 47:236-245.

18. Bendana, F.E., A.W. Galston, R. Kaur-Sawhney and P.J. Penny. 1965. Recovery of Labelled Ribonucleic Acid Following Administration of Labelled Auxin to Green Pea Stem Sections. Plant Physiol. 40:977-983.

19. Bergmeyer, H.U. and E. Bernt. 1965. Determination with Glucose Oxidase and Peroxidase. In 'Methods of Enzymatic Analysis'. ed. H.U. Bergmeyer. Academic Press, New York, London. p 123-130.

20. Biale, J.B. 1969. Metabolism at Several Levels of Organization in the Fruit of the Avacado, Persea Americana, Mill. Qual. Plant Mater. Veg. 19:141-153.

21. Biale, J.B. 1960. Respiration of Fruits. Encycl. Plant Physiol. 12:536-592.

22. Biale, J.B. and T. Solomos. 1974. Respiration and Fruit Ripening. In 'Facteurs et Regulation de la Maturation des Fruits'. Colloques Internationaux du Centre Nationale de la Recherche Scientifique, Paris. p 60.

23. Bonnichsen, R. 1965. Ethanol Determination with Alcohol Dehydrogenase and DPN. In 'Methods of Enzymatic Analysis. ed. H.U. Bergmeyer. Academic Press, New York, London. p 285-287.

24. Bown, A.N. and T. Aung. 1974. The Influence of 0.03% Carbon Dioxide on Protein Metabolism of Etiolated *Avena sativa* Coleoptiles. Plant Physiol. 54:19-22.

25. Bown, A.N., I.J. Dymock and T. Aung. 1974. A Synergistic Stimulation of *Avena sativa* Coleoptile Elongation by Indoleacetic Acid and Carbon Dioxide. Plant Physiol. 54:15-18.

26. Burg, S.P. 1973. Ethylene in Plant Growth. Proc. Nat. Acad. Sci. U.S.A. 70:591-597.

27. Burg, S.P. and M.J. Dijkman. 1967. Ethylene and Auxin Participation in Pollen Induced Fading of Vanda Orchid Blossoms. Plant Physiol. 42:1648-1650.

28. Burg, S.P. and C.O. Clagett. 1967. Conversion of Methionine to Ethylene in Vegetative Tissue and Fruits. Biochem. Biophys. Res. Commun. 27:125-130.

29. Burg, S.P. 1962. The Physiology of Ethylene Formation. Ann. Rev. Plant Physiol. 13:265-302.

30. Burg, S.P. and E.A. Burg. 1969. Interaction of Ethylene and Carbon Dioxide in the Control of Fruit Ripening. Qual. Plant Mater. Veg. 19:185-200.

31. Burg, S.P. and E.A. Burg. 1967. Molecular Requirements for Biological Activity of Ethylene. Plant Physiol. 42:144-152.

32. Burg, S.P. and E.A. Burg. 1965. Ethylene Action and Ripening of Fruits. Science. 148:1190-1196.

33. Burg, S.P. and E.A. Burg. 1964. Biosynthesis of Ethylene. Nature. 203:869-870.

34. Burg, S.P. and K.V. Thimman. 1961. The Conversion of Glucose-^{14}C to Ethylene by Apple Tissue. Arch. Biochem. Biophys. 95:450-475.

35. Burg, S.P. and K.V. Thimman. 1960. Studied on the Ethylene Production by Apple Tissue. Plant Physiol. 35:24-35.

36. Chadwick, A.V. and S.P. Burg. 1970. Regulation of Root Growth by Auxin-Ethylene Interaction. Plant Physiol. 45:192-200.

37. Chalutz, E., J.E. DeVay and E.C. Maxie. 1969. Ethylene Induced Isocoumarin Formation in Carrot Root Tissue. Plant Physiol. 44:235-241.

38. Chandra, G.R. and M. Spencer. 1962. Ethylene Production by Subcellular Particles from Tomatoes. Nature. 194:361-364.

39. Chibata, I. and T. Tosa. 1959. Studies of Amino Acids. XII. Studied on the Enzymatic Resolution (X): Acylase Activity in Plants. Bull. Agr. Soc. Japan. 28:370-376.

40. Chou, T.W. and S.F. Yang. 1973. The Biogenesis of Ethylene in *Penicillium digitatum*. Arch. Biochem. Biophys. 157:73-82.

41. Cleland, R. 1960. Ethionine and Auxin-Action in Avena Coleoptile. Plant Physiol. 35:585-588.

42. Crane, F.L. 1961. Structure and Function of Mitochondria. Ann. Rev. Plant Physiol. 12:13-34.

43. Curtis, R.W. 1969. Stimulation of Ethylene or Ethane Production by Malformin. Plant Cell Physiol. 10:909-916.

44. DeRobichon-Szulmajster, H. and Y. Surdin-Kerjan. 1971. Nucleic Acid and Protein Synthesis in Yeasts. Regulation of Synthesis and Activity. In 'The Yeasts', ed. A.H. Rose and J.S. Harrison. Academic Press, New York, London. Vol. 2, p 335-418.

45. Davies, E. and G.A. MacLachlan. 1968. Effects of Indoleacetic Acid on Intracellular Distribution of β-Glucanase Activities in the Pea Epicotyl. Arch. Biochem. Biophys. 128:595-600.

46. Demorest, D.M. and M.A. Stahman. 1971. Ethylene Production from Peptides and Protein Containing Methionine. Plant Physiol. 47:450-451.

47. Denny, F.E. 1924. Effect of Ethylene on Respiration of Lemons. Bot. Gaz. 77:322-329.

48. Drennan, C.H. 1974. University of Alberta: Personal Communication.

49. Durham, J.I., P.W. Morgan, J.M. Prescott and C.M. Lyman. 1972. Enzymatic Synthesis of Ethylene in Germinating Peanut Seed. Plant Physiol. 49:s-21.

50. Farber, E. 1973. ATP and Cell Integrity. Fed. Proc. 32:1534-1539.

51. Ferro, A.J. and K.D. Spence. 1973. Induction and Repression in S-adensylmethionine and Methionine Biosynthetic Systems of *Saccharomyces cerevisiae*. J. Bact. 116:812-817.

52. Frankel, C. and R. Dyck. 1973. Auxin Inhibition of Ripening in Bartlett Pears. Plant Physiol. 51:6-9.

53. Fuchs, Y. and E. Gertman. 1974. Studies of the Effects of Ethylene on Alcohol Dehydrogenase Activity. Plant Cell Physiol. 15: 701-708.

54. Ruchs, Y. and E. Gertman. 1973. Stabilization of Enzyme Activity in an Ethylene Atmosphere. Plant Cell Physiol. 14:197-199.

55. Fuchs, Y. and M. Lieberman. 1968. Effects of Kinetin, IAA and Gibberellin in Ethylene Production and Their Interactions in Growth of Seedlings. Plant Physiol. 43:2029-2036.

56. Galstone, A.W., S. Lavee, A. Seigel. 1968. The Induction and Repression of Peroxidase by 3-indole Acetic Acid. In 'Biochemistry and Physiology of Plant Growth Substances'. Runge Press, Ottawa. p 445-472.

57. Ghooprasert, P. 1971. Studies and Ethylene Biosynthesis from Acrylate, β-Alanine and L-Methionine. University of Alberta, Ph.D. Thesis.

58. Gibson, M.S. and R.E. Young. 1966. Acetate and Other Carboxylic Acids as Presursors of Ethylene. Nature. 210:529-530.

59. Giovanelli, J., L.D. Owens and S.H. Mudd. 1973. β-cystathionase *in vivo* Interaction by Rhizobitoxine and Role of the Enzyme in Methionine Synthesis in Corn Seedlings. Plant Physiol. 51: 492-503.

60. Giovanelli, J., L.D. Owens and S.H. Mudd. 1971. Mechanism of Inhibitions of Spinach β-cystathionase by Rhizobitoxine. Biochim. Biophys. Acta. 227:671-684.

61. Guillemant, P., G. Burkard, A. Steinmetz and J.M. Weil. 1973. Comparative Studies on tRNA$^{met}_{s}$ from Cytoplasm, Chloroplasts and Mitochondria of *Phaseolus vulgaris*. Plant Sci. Letters. 1:141-149.

62. Hackett, D.P., D.W. Haas, S.K. Griffiths and D.J. Neiderpruem. 1960. Studies on Development of Cyanide Resistant Respiration in Potato Tuber Slices. Plant Physiol. 35:8-19.

63. Hartman, C. 1969. Observation sur la participation du Cycle des Pentose Phosphates au Catabolisme Glucidique de Certains Fruits. Qual. Plant Mater. Veg. 19:67-77.

64. Herner, R.C. and K.C. Sink (Jr.) 1973. Ethylene Production and Respiratory Behaviour of the *rin* Tomato Mutants. Plant Physiol. 52:38-42.

65. Hutchinson, S.A. 1973. Biological Activities of Volatile Fungal Metabolites. Ann. Rev. Phytopathol. 11:223-246.

66. Ilga, L. and R.W. Curtis. 1968. Production of Ethylene by Fungi. Scinece. 159: 1357-1358.

67. Irvine, R.F. and D.J. Osborne. 1973. The effect of Ethylene on [1-^{14}C] Glycerol Incorporation into Phospholipids of Etiolated Pea Stems. Biochem. J. 136:1133:1135.

68. Jacobsen, D.W. and C.H. Wang. 1968. The Biogenesis of Ethylene in *Penicillium digitatum*. Plant Physiol. 43:1959-1966.

69. Kang, B.G., W. Newcomb and S.P. Burg. 1971. Mechanism of Auxin-Induced Ethylene Production. Plant Physiol. 47:504-509.

70. Kato, Y. and I. Uritani. 1972. Ethylene Biosynthesis in Diseased Root Tissue with Special Reference to the Methionine System. Agr. Biol. Chem. 36:2601-2604.

71. Kerting, D.L., R.E. Young and J.B. Biale. 1968. Effects of Monofluoroacetate on *Penicillium digitatum* Metabolism and on Ethylene Biosynthesis. Plant Cell Physiol. 9:617-631.

72. Key, J.K. and L. Ingle. 1968. RNA Metabolism in Response to Auxin. In 'Biochemistry and Physiology of Plant Growth Substances'. ed. F. Wightman and G. Setterfield. Runge Press, Ottawa. p. 711-722.

73. Kleglvic, D.B., B. Ladesic and M. Pokorny. 1968. Biochemical Studies in Tobacco Plants IV. N-Formylmethionine, a Metabolite of D-methionine in *Nicotina rustica*. Arch. Biochem. Biophys. 124: 443-449.

74. Klyne, M.A. 1974. Physiological and Biochemical Effects of Ethylene on Tulips. University of Alberta, M.Sc. Thesis.

75. Kobayashi, K. and T. Yamaki. 1972. Studies on Soluble RNA Binding Indoleacetic Acid in Etiolated Mung Bean Hypocotyl Sections. Plant Cell Physiol. 13:49-65.

76. Kotyk, A. and A. Kleinzeller. 1967. Affinity of the Yeast Membrane Carrier for Glucose and Its Role in the Pasteur Effect. Biochim. Biophys. Acta. 135:106-111.

77. Ku, H.S., H. Suge, L. Rappaport and H.K. Pratt. 1970. Stimulation of Rice Coleoptile Growth by Ethylene. Planta (Berl.) 90: 333-339.

78. Ku, H.S. and A.C. Leopold. 1970. Mitochondrial Responses to Ethylene and Other Hydrocarbons. Plant Physiol. 46:842-844.

79. Ku, H.S., S.F. Yang and H.K. Pratt. 1969. Ethylene Formation from α-Keto-γ-Methylthiobutyrate by Tomato Fruit Extracts. Phytochem. 8:567-575.

80. Ku, H.S. and H.K. Pratt. 1968. Active Mitochondria do not Produce Ethylene. Plant Physiol. 43:999-1001.

81. Kunkee, R.E. and M.A. Amerine. 1971. Yeasts in Wine-Making, in 'The Yeasts". ed. A.H. Rose and J.S. Harrison. Academic Press, New York, London. Vol. 3. p 5-71.

82. LaRue, T.A.G. and O.L. Gamborg. 1971. Ethylene Production by Plant Cell Cultures. Variations in Production During Growing Cycle and in Different Plant Species. Plant Physiol. 48:394-398.

83. Lieberman, M. and A.T. Kunishi. 1974. Ethylene-Forming Systems in Etiolated Pea Seedling Epicotyl Segments and in Apple Tissue. Plant Physiol. 53:s-17.

84. Lieberman, M., A.T. Kunishi and L.D. Owens. 1974. Specific Inhibitors of Ethylene Production in Fruit. In 'Facteurs et Regulation de la Maturation des Fruits'. Colloques Internationaux du Centre National de la Recherche Scientifique, Paris. p 44.

85. Lieberman, M. and A.T. Kunishi. 1971. An Evaluation of 4-S-Methyl-2-Keto-Butyric Acid as an Intermiediate in Biosynthesis of Ethylene. Plant Physiol. 47:476-580.

86. Lieberman, M., A. Kunishi, L.W. Mapson and D.A. Wardale. 1966. Stimulation of Ethylene Production in Apple Tissue Slices by Methionine. Plant Physiol. 41:376-382.

87. Lieberman, M., A.T. Kunishi, L.W. Mapson and D.A. Wardale. 1965. Ethylene Production from Methionine. Biochem. J. 97:449-459.

88. Lieberman, M.,and L.W. Mapson. 1964. Genesis and Biogenesis of Ethylene. Nature. 204:343-345.

89. Linnane, A.W. and J.M. Haslam. 1970. The Biogenesis of Yeast Mitochondria in 'Current Topics in Cellular Regulation'. ed. B.L. Horekar and E.K. Stadtman. Vol. 2 p 101-172.

90. Lor, K.L. and E.A. Cossins. 1972. Regulation of C_1 Metabolism by L-methionine in Saccharomyces cerevisiae. Biochem. J. 130: 773-783.

91. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall. 1951. Protein Measurement with the Folin Phenol Reagent. J. Biol. Chem. 193:265-275.

92. Lund, B.M. and L.W. Mapson. 1970. Stimulation by Erwina carotovora of Synthesis of Ethylene in Cauliflower Tissue. Biochem. J. 119:251-263.

93. Lynch, J.M. and S.H.T. Harper. 1974. Formation of Ethylene by Soil Fungus. J. Gen. Microbiol. 80:187-195.

94. Lynch, J.M. and S.H.T. Harper. 1974. Fungal Growth Rate and the Formation of Ethylene in Soil. J. Gen. Microbiol. 85: 91-96.

95. Lynch, J.M. 1973. Extracellular Ethylene Formation by Mucor hiemalis. J. Gen. Microbiol. 77:iv.

96. Lynch, J.M. 1972. Identification of Substrates and Isolation of Microorganisms Responsible for Ethylene Production in the Soil. Nature. 240:45-46.

97. Lyons, J.M. and H.K. Pratt. 1964. An Effect of Ethylene on Swelling of Isolated Mitochondria. Arch. Biochem. Biophys. 104:318-324.

98. Lyons, J.M., W.B. McGlasson and H.K. Pratt. 1962. Ethylene Production, Respiration and Internal Gas Concentrations in Cantaloupe Fruits at Various Stages of Maturity. Plant Physiol. 37:31-36.

99. McGlasson, W.B. 1969. Ethylene Production by Slices of Green Banana Fruit and Potato Tuber Tissue During the Development of Induced Respiration. Aust. J. Biol. Sci. 22:489-491.

100. McMurchie, E.J., W.B. McGlasson and I.L. Eaks. 1972. Treatment of Fruit with Propylene Gives Information About Biogenesis of Ethylene. Nature. 237:235-236.

101. MacLachlan, G.A., E. Davies and D.F. Fan. 1968. Induction of Cellulase by 3-indoleacetic Acid. In 'Biochemistry and Physiology of Plant Growth Substances'. ed. F. Wightman and G. Setterfield. Runge Press, Ottawa. p 443-454.

102. Maitra, P.K. and R.W. Estabrook. 1964. A fluorometric Method for the Enzymic Determination of Glycolytic Intermediates. Anal. Biochem. 7:472-484.

103. Maitra, P.K. and Z. Lobo. 1971. A Kinetic Study of Glycolytic Enzymes Synthesis in Yeast. J. Biol. Chem. 246:475-488.

104. Maitra, P.K. and Z. Lobo. 1971. Control of Glycolytic Enzyme Synthesis in Yeasts by Products of the Hexokinase Reaction. J. Biol. Chem. 246:489-499.

105. Malhotra, S.S. and M. Spencer. 1974. The Effects of Ethylene, Carbon Dioxide and Ethylene-Carbon Dioxide Mixtures on the Activity of 'Membrane-containing' and 'Highly Purified' Preparations of Adenosine Triphosphatase from Pea Cotyledon Mitochondria. Can. J. Biochem. (in press).

106. Mapson, L.W. and D.A. Wardale. 1971. Enzymes Involved in the Synthesis of Ethylene from Methionine, or Its Derivatives, in Tomatoes. Phytochem. 10:29-40.

107. Mapson. L.W., J.F. March, M.J.C. Rhodes and L.S.C. Wooltorton. 1970. A Comparative Study of the Ability of Methionine or Linolenic Acid to Act as Precursors of Ethylene in Plant Tissues. Biochem. J. 117:473-4[illegible].

108. Mapson, L.W. 1970. Biosynthesis of Ethylene and the Ripening of Fruit. Endeavour. 29;29-33.

109. Mapson, L.W., J.F. March and D.A. Wardale. 1969. Biosynthesis of Ethylene. 4-Methylmercapto-2-oxobutyric Acid: An Intermediate in the Formation from Methionine. Biochem. J. 115:653-661.

110. Mapson, L.W., R. Self and D.A. Wardale. 1969. Biosynthesis of Ethylene. Methanesulfinic Acid as Cofactor in the Enzymatic Formation of Ethylene from Methional. Biochem. J. 111: 413-418.

111. Mapson, L.W. 1969. Biogenesis of Ethylene. Biol. Rev. 44: 155-187.

112. Mapson, L.W. and D.A. Wardale. 1968. Biosynthesis of Ethylene. Enzymes Involved in Its Formation from Methional. Biochem. J. 107:433-442.

113. Mapson. L.A. and D.A. Wardale. 1967. Biosynthesis of Ethylene. Formation of Ethylene from Methional by a Cell-free Enzyme System from Cauliflower Floret. Biochem. J. 102:574-585.

114. Maw, G.A. 1966. Entry of Ethionine Sulphur into Yeast Protein. Biochem. J. 98:28P.

115. Meigh, D.F. 1960. Use of Gas Chromatography in Measuring Ethylene Production of Stored Apples. J. Sci. Food Agr. 11:381-385.

116. Meigh, D.F., J.D. Jones and A.C. Hulme. 1967. The Respiration Climacteric in the Apple. Production of Ethylene and Fatty Acids in Fruit Attached to and Deteched from the Tree. Phytochem. 6:1507-1515.

117. Mehard, C.W. and J.M. Lyons. 1970. A Lack of Specificity for Ethylene-Induced Mitochondrial Changes. Plant Physiol. 46:36-39.

118. Meheruik, M. and M. Spencer. 1967. Studies on Ethylene Production by a Subcellular Fraction from Ripening Tomatoes - I. Phytochem. 6:535-543.

119. Meheruik, M. and M. Spencer. 1967. Studies on Ethylene Production by a Subcellular Fraction from Ripening Tomatoes - II. Phytochem. 6:545-549.

120. Meheruik, M. and M. Spencer. 1964. Effects of Nitrogen and of Respiratory Inhibitors on Ethylene Production by a Sub-cellular Fraction from Tomatoes. Nature. 204:43-45.

121. Merck Index. 1968. ed. P.G. Stecher, 8th Edition. p 311, 675.

122. Murata, T. and T. Minamide. 1970. Studies on Organic Acid Metabolism and Ethylene Production During Controlled Atmosphere Storage of Apples (Mallus pumila Miller cv rolls). Plant Cell Physiol. 11:857-863.

123. Naider, F., J.M. Becker and E. Katzir-Katchalski. 1974. Utilization of Methionine-Containing Peptides and Their Derivatives by a Methionine-Requiring Auxotropic Saccharomyces cerevisiae. J. Biol. Chem. 249:9-20.

124. Nord, F.F. and J. Weichherz. 1929. Enzymic Process in Germinating Barley. Chem. Abstr. 23:5476.

125. Nord, F.F. and J. Weichherz. 1929. The Mechanism of Enzyme Action. The Relation Between Enzyme Action and Adsorption. Chem. Abstr. 23:5475-5476.

126. Nord, F.F. and K.W. Franke. 1928. On the Mechanism of Enzyme Action II. Further Evidence Confirming the Observations that Ethylene Increases the Permeability of Cells and Act as Protector. J. Biol. Chem. 79:27-51.

127. Negm, F.B., O.E. Smith and J. Kumanoto. 1972. Interaction of Carbon Dioxide and Ethylene in Overcoming Thermodormancy in Lettuce Seeds. Plant Physiol. 49:869-872.

128. Olson, A.O. and M. Spencer. 1968. Studies on the Mechanism of Action of Ethylene I. The Effect of Ethylene on Mitochondria Prepared from Bean Cotyledons. Can. J. Biochem. 46:277-282.

129. Olson, A.O. and M. Spencer. 1968. Studies on the Mechanism of Action of Ethylene II. Effects of Ethylene on Mitochondria from Rat Liver and Yeast, and on Mitochondrial Adenosine Triphosphatase. Can. J. Biochem. 46:283-288.

130. Osborne, D.J. 1968. Ethylene as a Plant Hormone. In 'Plant Growth Regulators. Society of Chemical Industry Monograph No. 31. p 236-250.

131. Owens, L.D., J.F. Thompson, R.G. Pitcher and T. Williams. 1972. Structure of Rhizobitoxine, a Toxic Enol Ether Amino Acid from Rhizobium japonicum. J. Chem. Soc. Chem. Commun. p 714.

132. Owens, L.D., M. Lieberman and A. Kunishi. 1971. Inhibition of Ethylene Production by Rhizobitoxine. Plant Physiol. 48:1-4.

133. Owens, L.D., S. Guggenheim and J. Hilton. 1968. Rhizobium Synthesized Phytotoxin: An Inhibitor of β-cystathionase in Salmonella typhimurium. Biochim. Biophys. Acta. 158:219-225.

134. Phan, C.T. 1971. L'Ethylene Metabolism et Activite Metabolique. Masson and Cie. Paris, p 43-48.

135. Phan, C.T. 1970. Conversion of Various Substrates to Ethylene by Flowers. Physiol. Plant. 23:981-984.

136. Phan, C.T. 1962. Contribution a l'etude de la production de l'ethylene par le Penicillium digatatum, Sacc. Rev. Gen. Bot. 60:505-543.

137. Phan, C.T. 1962. Observation sur l'Emission d'Ethylene par les Fruits. Advan. Hort. Sci. Appl. 2:238-241.

138. Philips, D.R. 1971. Pea Mitochondrial Adenosine Triphosphatase Activity and Ethylene. University of Alberta, Ph.D. Thesis.

139. Porutskii, G.V., A.S. Luchko and K.I. Matkovskii. 1962. Sov. Plant Physiol. 9:382-384. Cited by F.B. Abeles in 'Biosynthesis and Mechanism of Action of Ethylene'. Ann. Rev. Plant Physiol. 1972. 23:259-292.

140. Pratt, H.K. and J.D. Goeschl. 1969. Physiological Role of Ethylene in Plants. Ann. Rev. Plant Physiol. 20:541-584.

141. Racker, E. 1965. Mechanisms in Bioenergetics. Academic Press, New York and London. p 198.

142. Rahiala, E.L., M. Kekomaki, J. Janne, A. Raina and N.R.C. Raina. 1971. Inhibition of Pyridoxal Enzymes by L-Canaline. Biochim. Biophys. Acta. 227:337-343.

143. Ramchandra, G., M. Spencer and M. Meheriuk. 1963. Evolution of Ethylene by Sub-Cellular Particles from Tomatoes as Influenced by Components of the System. Nature. 199:767-769.

144. Ratner, A., R. Goren and S.P. Monselise. 1969. Activity of Pectin Esterase and Cellulase in the Abscission Zone of Citrus Leaf Extracts. Plant Physiol. 44:1717-1723.

145. Rappaport, L., I. Rylski and H.K. Pratt. 1974. Effects of Carbon Dioxide and Ethylene in Terminating Dormancy in Potato Tubers. Plant Physiol. 53:s-6.

146. Reid, M.S. and H.K. Pratt. 1972. Effects of Ethylene on Potato Tuber Respiration. Plant Physiol. 49:252-255.

147. Rhodes, M.J.C., L.S.C. Wooltorton and A.C. Hulme. 1969. Some Enzyme Systems in Ripening of Apples. Qual. Plant Mater. Veg. 19:167-183.

148. Ridge, I. and D.J. Osborne. 1970. Role of Peroxidase when Hydroxyproline-Rich Protein in Plant Cell Walls is Increased by Ethylene. Nature New Biol. 299:205-208.

149. Rothstein, A. and M. Bruce. 1958. The Efflux of Potassium From Yeast Cells into a Potassium Free Medium. J. Cell Comp. Physiol. 51:439-455.

150. Ryan, J.P. and H. Ryan. 1972. The Role of Intracellular pH in the Regulation of Cation Exchanges in Yeast. Biochem. J. 128: 139-146.

151. Ryan, H. J.P. Ryan and W.H. O'Conner. 1971. The Effect of Diffusible Acids on Potassium Ion Uptake by Yeast. Biochem. J. 125:1081-1085.

152. Sacher, J.A. and S.O. Salminen. 1969. Comparative Studies of Effect of Auxin and Ethylene on Permeability and Synthesis of RNA and Protein. Plant Physiol. 44:1371-1377.

153. Sakai, S. and H. Imaseki. 1971. Auxin-Induced Ethylene Production by Mung Bean Hypocotyl Sections. Plant Cell Physiol. 12: 349:359.

154. Sarkar, S.K. 1972. Ethylene and Phenol Metabolism in Stored Carrots. University of Alberta, Ph.D. Thesis.

155. Sarkissian, I.V. and R.G. McDaniel. 1966. Regulation of Mitochonrial Activity by Indoleacetic Acid. Biochim. Biophys. Acta. 128:413-418.

156. Shaw, F.H. 1936. The Mechanism of the Action of Ethylene on Cell Processes. Aust. J. Exp. Biol. 13:95-102.

157. Shimoda, C. and N. Yanagishima. 1968. Strain Dependence of the Cell-Expanding Effect of β-1,3-Glucanase in Yeast. Physiol. Plant. 21:1163-1169.

158. Shimokava, K. and Z. Kasai. 1966. Biogenesis of Ethylene in Apple Tissue. I. Formation of Ethylene from Glucose, Acetate, Pyruvate, and Acetaldehyde in Apple Tissue. Plant Cell Physiol. 7:1-9.

159. Smith, D.E. and F.B. Negm. 1974. Effects of Ethylene and Carbon Dioxide on Germination of Osmotically-inhibited Lettuce Seeds. Plant Physiol. 53:5-6.

160. Solomos, T. and G.G. Laties. 1975. The Mechanism of Ethylene and Cyanaide Action in Triggering the Rise in Respiration in Potato Tubers. Plant Physiol. 55:73-78.

161. Solomos, T. and G.G. Laties. 1975. The Diversion by Ethylene of Respiratory Electrons to the Cyanide Resistant Path. Plant Physiol. (in press).

162. Solomos, T. and G.G. Laties. 1973. Cellular Organization and Fruit Ripening. Nature 245:390-392.

163. Spencer, M. 1969. Ethylene in Nature. In 'Forschritte d. Chem. Org. Naturst'. XXVII:31-80.

164. Spencer, M. 1959. Production of Ethylene by Mitochondria from Tomatoes. Nature. 184:1231-1232.

165. Spencer, M. and M. Meheriuk. 1963. Influence of Temperature and Aging on Ethylene Production by a Sub-Cellular Fraction from Toromtoes. Nature. 199:1077-1078.

166. Splittstoesser, W.E. 1966. Dark Carbon Dioxide Fixation and Its Role in the Growth of Plants. Plant Physiol. 41:755-759.

167. Sprawberry, B.A., W.C. Hall and C.S. Miller 1965. Biogenesis of Ethylene in Penicillium digitatum. Nature. 208:1322-1323.

168. Steen, D.A. and A.V. Chadwick. 1973. Effects of Cycloheximide on Indoleacetic Acid-Induced Ethylene Production in Pea Root Tips. Plant Physiol. 52:171-173.

169. Stinson, R.A. and M. Spencer. 1969. β-alanine as an Ethylene Precursor. Investigations Towards Preparation and Properties, of a Soluble Enzyme System From a Subcellular Particulate Fraction of Bean Cotyledons. Plant Physiol. 44:1217-1226.

170. Stinson, R.A. and M. Spencer. 1970. Respiratory Control, Oxidative Phosphorylation, Respiration, Rate of ATP Hydrolysis, and Ethylene Evolution in Subcellular Particulate Fractions from Cotyledons of Germinating Seedlings. Can. J. Biochem. 48: 541-546.

171. Stinson, R.A. 1968. Ethylene Biosynthesis by Enzyme Systems from Bean Cotyledons. University of Alberta, Ph.D. Thesis.

172. Stickland, L.H. 1956. Endogenous Respiration and Polysaccharide Reserves in Bakers Yeast. Biochem. J. 64:498-503.

173. Stickland, L.H. 1956. Pasteur Effect in Normal Yeast and Its Inhibition by Various Agents. Biochem. J. 64:503-515.

174. Suomalainen, H. and E. Oura. 1971. Yeast Nutrition and Solute Uptake. In 'The Yeasts'. ed. A.H. Rose and J.S. Harrison. Academic Press, New York and London. Vol. 2. p 3-74.

175. Tager, J.M. 1956. The Role of Pentose Cycle in the Ripening Banana. South Afri. J. Sci. 53:167-170.

176. Tanimoto, E. and Y. Masuda. 1968. Effect of Auxin on Cell Wall Degrading Enzymes. Physiol. Plant. 24:802-826.

177. Thompson, J.E. and M. Spencer. 1967. Ethylene Production from β-alanine by an Enzyme Powder. Can. J. Biochem. 45:563-571.

178. Wang, C.H. 1967. Radiorespirometry. In 'Methods of Biochemical Analysis'. ed. D. Glick, Interscience. Vol. 15. p 311-368.

179. Wang, C.H. A. Persyn and J. Krackov. 1962. Role of the Krebs Cycle in Ethylene Biosynthesis. Nature. 195:1306-1308.

180. Westley, J. 1969. Enzymic Catalysis. Harper and Row Publishers, New York. p 56.

181. Whitfield, C.F. and H.E. Morgan. 1973. Effect of Anoxia on Sugar Transport in Avian Erythrocytes. Biochim. Biophys. Acta. 307:181-196.

182. Wolf, J. 1969. The Oxygen Exchange in Fruits and Its Relationship to Ripening, Aging and Storage. (Article in German, Abstract in English). Qual. Plant Mater. Veg. 19:79-127.

183. Woltz, S.S. 1963. Growth Modifying Antimetabolite Effects of Amino Acids on Crysanthemum. Plant Physiol. 38:93-99.

184. Yang, S.F. 1974. Ethylene Biosynthesis in Fruit Tissues. In 'Facteurs et Regulation de la Maturation des Fruits.' Colloques Internationaux du Centre National de la Recerche Scientifique, Paris.

185. Yang, S.F. and A.H. Baur. 1969. Pathways of Ethylene Biosynthesis. Qual. Plant Mater. Veg. 19:201-220.

186. Yang, S.F. 1967. Biosynthesis of Ethylene. Ethylene Formation from Methional by Horseradish Peroxidase. Arch. Biochem. Biophys. 122:481-487.

187. Zimmerman, P.W. and F. Wilcoxon. 1935. Several Chemical Growth Substances Which Cause Initiation of Roots and Other Responses in Plants. Contrib. Boyce Thompson Inst. 7:209-229.